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Antoine Nkuba Ndaye

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THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

Microbiologie, Maladies Transmissibles et Hygiène

École doctorale : Sciences Chimiques et Biologiques pour la Santé (CBS2)

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(IRD UMI 233 – INSERM U1175 – Université de Montpellier)

EMERGING VIRUSES IN THE DEMOCRATIC REPUBLIC OF THE CONGO: CHARACTERIZATION OF THE HUMORAL IMMUNE RESPONSE AGAINST EBOLA VIRUS AND SARS- COV-2

Présentée par Antoine Nkuba Ndaye
Le 15 décembre 2022

Sous la direction de Martine Peeters et de Steve Ahuka Mundeke

Devant le jury composé de

Nathalie Chazal, Professeur des Universités, Faculté de Médecine de Montpellier, France

Désiré Mashinda Kulimba, Professeur Full, Université de Kinshasa, RD Congo

Simon François, Professeur Emérite, Faculté de Médecine - Université de Paris Diderot, France

Jean-Jacques Muyembe Tamfum, Professeur Ordinaire, Université de Kinshasa, RD Congo

Steve Ahuka Mundeke, Professeur Ordinaire, Université de Kinshasa, RD Congo

Martine Peeters, Directeur de Recherche, IRD, Montpellier, France

Présidente

Rapporteur

Rapporteur

Examineur

Co-Directeur de thèse

Directeur de thèse



UNIVERSITÉ
DE MONTPELLIER

ABSTRACT

The frequency of Ebola virus outbreaks has been increasing since 2017, resulting in an increase in the number of known survivors treated with specific molecules and others not identified by response teams in an emergency setting of new viruses such as SARS-CoV-2.

We have shown that people discharged after two RT-PCR tests could be Ebola patients who presented with a pauci-symptomatic form and who presented after the viraemic phase. We have also shown that the production of anti-Ebola antibodies in people treated with anti-Ebola molecules appears to be delayed and that antibody levels drop very rapidly over time. Contrary to official reports on the number of confirmed COVID-19 cases in the Democratic Republic of the Congo, we have reported a very wide spread of SARS-CoV-2 in Kinshasa following the first wave of the pandemic.

This work, conducted in the context of emerging disease studies, demonstrates on the one hand the importance of serology for understanding the evolution of newly identified or previously known emerging diseases and, on the other, the importance to contribute with studies in survivors of Ebola virus disease to prevent relapse, which could be possible factors for disease emergence.

KEY WORDS: Ebola virus, SARS-CoV-2, Antibody, EBOV treatment, DRC.

TITRE : Les virus émergents en République Démocratique du Congo : caractérisation de la réponse immunitaire humorale contre Ebola et le SARS-CoV-2.

RESUME

La fréquence des épidémies de la maladie à virus Ebola est de plus en plus élevée depuis 2017 avec comme conséquence l'augmentation du nombre des survivants connus et traités par des molécules spécifiques et d'autres non identifiés par des équipes de riposte dans un contexte d'émergence de nouveaux virus tel que le SARS-CoV-2.

Nous avons démontré que des personnes déchargées après deux tests RT-PCR pourraient être des patients Ebola ayant présenté une forme pauci-symptomatique et qui se sont présentés après la phase virémique. Nous avons aussi démontré que la production des anticorps anti-Ebola chez des personnes traitées avec des molécules anti-Ebola semble être tardive et les taux d'anticorps déclinent très rapidement au fil du temps. Contrairement aux rapports officiels sur le nombre de cas confirmés de COVID-19 en RDC, nous avons rapporté une diffusion très grande du SARS-CoV-2 à Kinshasa après la première vague de la pandémie.

Ces travaux réalisés dans le cadre des études sur les émergences montrent d'une part l'importance de la sérologie dans la compréhension de l'évolution des maladies émergentes nouvellement identifiées ou anciennement connues et d'autre part, l'importance de continuer des études chez des survivants de la maladie à virus Ebola pour prévenir des cas de relapse qui peuvent être des facteurs d'émergence potentiels.

Mots-clés : Ebola virus, SARS-CoV-2, Anticorps, traitement EBOV, RDC.

DISCIPLINE : Microbiologie, Maladie Transmissibles et Hygiène

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911 Avenue Agropolis, BP 64501, 34394 Montpellier, cedex 5, France

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List of abbreviations and acronyms

AIDS	: Acquired immunodeficiency syndrome
ASGP-R	: Asialoglycoprotein receptor
BDBV	: Bundibungyo ebolavirus
BSL-4	: Biosafety Level 4
cDNA	: Complementary deoxyribonucleic acid
CLECs	: C-type lectins
CLIA	: Chemiluminescence immunoassays
CRDs	: Carbohydrate recognition domains
cRNA	: Complementary positive stranded RNA
C _T	: Cycle threshold
DALYS	: Disability-adjusted life years
DNA	: Deoxyribonucleic acid
DRC	: Democratic Republic of the Congo
EBOV	: Ebolavirus
EIA	: Alinity Enzyme immunoassay
EIDs	: Emerging Infectious Diseases
ELISA	: enzyme-linked immunosorbent assay
ETC	: Ebola Treatment Centers
EVD	: Ebola virus disease
FDA	: United States Food and Drug Administration
GP	: Glycoprotein
gRNA	: Genomic ribonucleic acid
GTPases	: Guanosine Triphosphate-ases
HIV	: Human immunodeficiency virus
HZ	: Health zone
I.F.A	: Indirect immunofluorescence assay
IBB	: Institut Bouisson Bertrand
ICTV	: International Committee on Taxonomy of Viruses
IFNs	: Interferons
IL8	: Interleukin 8
INAAT	: Isothermal Nucleic acid amplification test
INRB	: Institut National de Recherche Biomédicale
IPCC	: Intergovernmental panel on climate change
LFIA	: Lateral flow immunoassays
MOH	: Ministry of Public Health
MSD	: Hydrophobic membrane-spanning domain
N7 Mtase	: N-seven methyltransferase
NAAT	: Nucleic acid amplification test
NCP1	: Niemann-Pick Disease, Type C1
NGS	: Next-generation sequencing
NGS	: Next-generation sequencing

NO	: Nitric oxide
NP	: Nucleoprotein
nsp	: Nonstructural protein
nsps	: Non-structural proteins
PCR	: Polymerase chain reaction
PNSPG	: Programme National de Suivi des Personnes Guéries
PoC	: Point of care
POC	: Point-of-care
PS	: Phosphatidylserine
R ₀	: Basic reproductive number
RBD	: Receptor binding domain
RBD	: Binding receptor
RBM	: Receptor binding motif
RdRP	: RNA-dependent RNA polymerase
RESTV	: Reston ebolavirus
RNA	: Ribonucleic acid
RNP	: Ribonucleio-protein
RT-PCR	: Reverse Transcription Polymerase Chain Reaction
SARS	: Severe Acute Respiratory Sybdrom
SARS-CoV-2	: Severe Acute Respiratory Syndrome-Coronavirus 2
SFV	: Semliki Forest virus
sGP	: Soluble glycoprotein
sgRNA	: Subgenomic ribonucleic acids
SHFV	: Simian hemorrhagic fever virus
ssGP	: Second poorly soluble glycoprotein
STAU1	: Staufen1
SUDV	: Sudan ebolavirus
TAFV	: Taï-forest ebolavirus
TATase	: 3'-terminal adenylyltransferase
TMPRSS2	: Transmembrane serine protease 2
TNF- α	: Tumor necrosis factor alpha
TOP1	: Topoisomerase 1
TPC2	: Two-pore channel
TRAIL	: Tumor necrosis factor alpha related apoptosis-inducing ligand
USA	: United States America
USAMRIID	: United States Army Medical Research Institute of Infectious Diseases
UTR	: Untranslated region
VLPs	: Virla-like particles
VOC	: Variant of concern
VOI	: Variant of interest
VP24	: Viral protein 24
VP30	: Viral protein 30

VP35 : Viral protein 35
VP40 : Viral protein 40
VUM : Variant under monitoring
WHO : World Health Organization
ZEBOV : Zaire ebolavirus

Résumé des travaux en Français

Les maladies infectieuses émergentes sont définies comme étant des maladies qui sont nouvellement reconnues dans une population ou qui ont existé mais dont l'incidence ou la portée géographique augmente rapidement. Dans ce groupe nous retrouvons les maladies comme le syndrome d'immunodéficience acquise (SIDA), le syndrome respiratoire aigu sévère (SRAS), et la maladie à virus Ebola (MVE) pour ne citer que celles-là. D'après le rapport du Center for Disease Control and Prevention (CDC), environ 75% des pathogènes responsables de ces émergences sont d'origine zoonotique et la plupart sont des virus. Les agents pathogènes émergents ont évolué pendant des siècles et ont provoqué plusieurs épidémies et pandémies qui ont entraîné des millions de décès et d'invalidités dans le monde. Par conséquent, la détection précoce, le traitement des patients et, si nécessaire, la mise en quarantaine des patients sont des outils de santé publique pour les combattre. Au cours des dernières décennies, la médecine a réalisé plusieurs avancées scientifiques, notamment dans le domaine du diagnostic des maladies infectieuses. Cela comprend, par exemple, le développement de méthodes moléculaires telles que la polymérase chain reaction (PCR) et le séquençage, qui ont une sensibilité et une spécificité améliorées, qui sont des atouts pour une bonne détection et caractérisation des micro-organismes. La lutte contre les épidémies virales comprend notamment la compréhension de la diffusion des pathogènes dans une population donnée, l'évaluation de l'état immunitaire dans la communauté, du vaccin et des traitements utilisés. La détection des anticorps et leur caractérisation par des méthodes sérologiques permet une meilleure compréhension des maladies émergentes et de leur dynamique afin de mieux adapter les mesures de contrôle des infections à chaque pathogène spécifique.

La MVE, apparue pour la première fois lors de deux épidémies simultanées à Nzara, au Soudan, et à Yambuku, en RDC, est une virose très meurtrière, avec un taux de mortalité atteignant 90%. En effet, cette maladie pose un problème majeur de santé publique en Afrique sub-saharienne. En général, le cas index de la MVE s'infecte par contact avec des animaux infectés (généralement lors de l'abattage, de la préparation ou de la consommation) et les infections interhumaines ultérieures sont généralement causées par le contact avec des fluides biologiques provenant des personnes infectées. La plupart des épidémies de MVE se sont produites principalement en Afrique centrale, avec un nombre limité de victimes

dans des zones reculées. Cependant, entre 2014 et 2016, la MVE est apparue dans des villes de trois pays d'Afrique de l'Ouest, touchant plus de 28,000 personnes et tuant plus de 11,000. La fréquence des flambées a augmenté ces dernières années, y compris en RDC, où 7 épidémies consécutives ont été enregistrées à Likati (nord-est), Equateur (nord-ouest) et dans les provinces du Nord-Kivu, du Sud-Kivu et de l'Ituri (nord-est) entre 2017 et 2022. La 10^{ème} épidémie de MVE au Nord-Kivu, au Sud-Kivu et en Ituri a été la plus importante et la plus difficile à contrôler jamais enregistrée en RDC, entraînant 3,470 cas et 2,287 décès. La réticence de la population à se tourner vers les Centres de Traitement Ebola (CTE) et les violences contre les équipes et structures de riposte ont entravé le bon déroulement du travail de terrain et facilité la propagation de la maladie.

Dans le cadre de cette thèse, nous avons évalué l'immunité humorale contre le virus Ebola chez l'homme dans deux principales populations d'étude : premièrement, nous avons considéré les patients suspects MVE mais diagnostiqués comme non-cas après deux tests RT-PCR négatifs au cours de la 10^{ème} épidémie de MVE de RDC. Pour cette étude, notre question de recherche était de savoir combien de patients déchargés comme non-cas seraient des cas oubliés de MVE ? L'objectif poursuivi était de déterminer la prévalence des anticorps anti-Ebola chez des patients suspects d'Ebola mais diagnostiqués comme négatifs après deux tests RT-PCR négatifs. Deuxième, l'évaluation de la concentration des anticorps chez des personnes guéries d'Ebola a été faite à l'aide d'une étude de cohorte rétrospective sur des échantillons des patients guéris d'Ebola qui étaient suivis dans le cadre du projet « les vainqueurs d'Ebola ». En effet, lors de la 10^{ème} épidémie de MVE, des nouveaux traitements spécifiques pour EBOV avaient été utilisés à la fois suivant le protocole d'utilisation compassionnelle (MEURI) et plus tard dans un essai clinique randomisé. De novembre 2018 au 9 août 2018, 4 centres ont participé à des essais cliniques randomisés (Mangina, Beni, Butembo et Katwa) avec un total de 681 participants. Quatre molécules ont été utilisées dont trois anticorps monoclonaux (ZMapp, Inmazed et Ansuvimab) et un vuricide (Remdesivir). Cependant, Inmazed et Ansuvimab avaient montré une bonne efficacité et ont été utilisées pour le traitement de nouveaux patients atteints de MVE. De façon générale les traitements administrés ont amélioré la survie des patients avec MVE. Cependant leur utilisation soulève plusieurs questions notamment celle en rapport avec leur impact sur la dynamique des anticorps chez des survivants. Ainsi, cette étude avait pour objectif d'évaluer l'impact des traitements anti-Ebola sur la dynamique des anticorps chez

des personnes guéries d'Ebola en comparaison avec la cohorte des guéries non traitées de Guinée.

La deuxième partie de la thèse a été axée sur la détermination de la séroprévalence du Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), récemment identifié en Chine après avoir probablement émergé du marché de Wuhan en décembre 2019. La COVID-19, maladie causée par le SARS-CoV-2 a été déclarée comme pandémie mondiale par l'OMS le 10 mars 2020. En effet, la RDC avait rapporté son premier cas de COVID-19 le 10 mars 2020 à Kinshasa, la capitale. L'évolution de la pandémie en Afrique en général et en RDC en particulier a pour sa part, soulevé de nombreuses questions qui ont nécessité des investigations approfondies. Par exemple, une étude d'estimation mathématique avait prédit plus de 70,000,000 de cas et plus de 3,000,000 pendant la première année de circulation du virus en Afrique. Cependant, six mois après la déclaration de la pandémie de COVID-19 en RDC, et après la première vague, seuls 11,078 cas confirmés de COVID-19 ont été signalés, avec 303 décès dans le pays pour une population d'environ 90,000,000 d'habitants. Ces nombres de cas et de décès déclarés étaient bien inférieurs à ceux prévus, suggérant une hétérogénéité. Ainsi, l'Organisation Mondiale de la Santé (OMS) avait recommandé des enquêtes de séro-surveillance en population générale pour compléter les chiffres rapportés par les pays Africains et estimer l'étendue réelle de la maladie afin de prendre des mesures de santé publique nécessaires. Plusieurs études sérologiques ont été menées en Afrique et ont montré une grande variabilité de la séroprévalence du SARS-CoV-2 dans différents pays, mais la plupart ont été menées dans des populations spécifiques telles que les donneurs de sang, les professionnels de la santé ou d'autres populations à haut risque. En plus des facteurs épidémiologiques, les différences de séroprévalence signalées peuvent s'expliquer par le fait que la plupart des tests sérologiques utilisés détectent des anticorps dirigés contre un seul antigène à la fois. Pour notre part, l'objectif était de développer et évaluer le test sérologique de détection des anticorps contre le SARS-CoV-2 chez l'homme et de déterminer la prévalence des anticorps IgG anti-SARS-CoV-2 en population générale à Kinshasa.

Comme résultats, nous avons rapporté dans la première partie de la thèse consacrée au virus Ebola une prévalence de 2,3 % des patients positifs à au moins deux antigènes du virus Ebola et avons estimé qu'environ 1,314 infections à EBOV non diagnostiquées pourraient

avoir eu lieu au cours de la 10^{ème} épidémie de MVE en RDC parmi les cas suspects de MVE déclarés négatifs après deux tests RT-PCR consécutifs. En fait, EBOV est connu pour provoquer une maladie grave chez l'homme et d'autres animaux tels que les antilopes, les singes et les porcs rendant anecdotique les suggestions de forme asymptomatique ou paucisymptomatique de MVE pendant plusieurs années. Cependant, des données récemment publiées ont mis en évidence une possible infection non identifiée chez les contacts asymptomatiques ou paucisymptomatiques de cas confirmés de MVE. Par exemple, les études menées lors de l'épidémie d'Ebola en Afrique de l'Ouest entre 2014 et 2016 ont rapporté une séro-prévalence des anticorps anti-Ebola chez des contacts des cas confirmés. Nos résultats ont été obtenus en utilisant des critères de positivité restrictifs basés notamment sur le profil des survivants guinéens, où la positivité a été déterminée par la réactivité d'un sujet à au moins deux des trois antigènes du virus Ebola inclus dans notre panel d'analyse qui contient la nucléoprotéine, la glycoprotéine et la protéine virale 40. Ces résultats renforcent nos connaissances et notre compréhension de la clinique asymptomatique ou paucisymptomatique de l'infection par le virus Ebola et démontrent davantage l'évolution et l'adaptation du virus Ebola au fil du temps. Il devient de plus en plus clair qu'une épidémie de MVE peut partir d'une personne guérie porteuse du virus quiescent dans des sites immunologiques privilégiés comme l'humeur aqueuse ou les testicules. De récents rapports génomiques sur des cas de rechute de la MVE en Guinée et en RDC en sont des exemples. Dans ce contexte, l'analyse sérologique peut être utilisée pour évaluer les cas de MVE oubliés parmi les suspects de MVE qui se sont révélés négatifs après le test RT-PCR, mais aussi les contacts familiaux des cas confirmés de MVE qui ont développé ou non des symptômes. Par conséquent, les personnes testées positives pour la MVE par des tests sérologiques doivent être suivies pour prévenir la récurrence de l'EBOV et la transmission possible du virus à d'autres personnes. Ainsi, il est important de sélectionner avec soin le test à utiliser et en particulier les critères de positivité qui peuvent optimiser la sensibilité et la spécificité pour EBOV.

Dans le suivi longitudinal des survivants, nous avons évalué la concentration des anticorps parmi les survivants de la MVE qui avaient reçu des traitements spécifiques dont trois anticorps monoclonaux (ZMapp, Ansumab et Inmazeb) et un virucide (Remdesivir). En effet, nous avons observé un pourcentage élevé (23,7%) de participants séronégatifs pour au moins deux antigènes à la sortie des CTEs alors que plusieurs études qui ont décrit

l'histoire naturelle de la MVE ont rapporté que les anticorps d'isotype G (IgG) sont détectés précocement, généralement dans la première semaine après le début des symptômes. Par conséquent, les traitements spécifiques reçus peuvent être la cause de la production tardive d'anticorps que nous avons observée. Il est donc extrêmement important d'évaluer le statut immunitaire des survivants traités lorsqu'ils quittent les CTEs et d'assurer un suivi régulier pour détecter une éventuelle persistance virale afin de réduire le risque de rechute à court terme, comme cela s'est produit en RDC lors de la 10^{ème} épidémie de MVE. De plus, des taux d'anticorps anormalement bas ont été observés chez deux survivants de la MVE de la 10^{ème} épidémie en RDC qui ont développé une méningite virale après avoir été guéris et ces survivants avaient tous été traités avec des anticorps monoclonaux lors du premier épisode de l'infection à EBOV.

Par rapport aux survivants de Guinée, notre cohorte de survivants a montré une baisse rapide des anticorps au fil du temps avec une probabilité significativement plus faible de rester positif séropositif aux antigènes EBOV au cours du temps dans tous les groupes de traitement mais la baisse est plus rapide dans le groupe Ansuvimab. En effet, nous pensons que les traitements reçus peuvent être à l'origine de cette baisse rapide des anticorps dans notre cohorte, même si nous ne pouvons pas exclure l'influence d'autres facteurs tels que l'âge, la charge virale en phase aiguë et, chez certains d'entre eux, le vaccin. Des expériences sur des animaux ont montré que la production d'anticorps peut être perturbée par une immunisation passive. Chez la souris, il a été suggéré que les anticorps maternels inhibent la production d'anticorps en internalisant les anticorps maternels par les cellules présentatrices d'antigène et en supprimant les épitopes antigéniques des lymphocytes B. Chez les macaques, les thérapies par anticorps monoclonaux ont été associées à la persistance du virus Ebola dans les cellules nerveuses. Des études menées sur des humains ont montré la même tendance, par exemple une étude de cohorte rétrospective aux États-Unis chez des patients atteints de COVID-19 traités avec des anticorps monoclonaux a montré une inhibition de la réponse humorale endogène. Dans le contexte des épidémies récurrentes de MVE en Afrique en général et en RDC en particulier, les survivants jouent un rôle important dans ces rechutes. Nos résultats sont très importants car ils soulignent la nécessité de continuer à prendre en charge les survivants de la MVE, non seulement pour faciliter leur réinsertion dans la communauté, mais aussi pour établir un calendrier de suivi clinique, biologique et psychologique.

Dans la deuxième partie consacrée au SARS-CoV-2, nous avons rapporté une séroprévalence de SARS-CoV-2 de 16,6% et avons estimé qu'environ 2,426,406 infections avaient eu lieu au cours des six premiers mois de circulation du virus dans la ville de Kinshasa. Ces résultats faisaient partie des premières études de séroprévalence à être menées en population générale dans les pays africains, où le nombre de cas confirmés déclarés était très faible par rapport aux autres continents mais aussi par rapport aux modèles de prédiction qui annonçaient plus de 75 millions de cas au cours de la première année de circulation du virus. Cependant, d'autres enquêtes au cours de la même période ont rapporté des fréquences extrêmement variables, parfois avec une prévalence incroyablement faible et d'autres fois avec une prévalence très élevée. Cette variation peut être causée par le fait que la majorité de ces enquêtes ont été menées dans des populations spécifiques, mais cela peut également être dû au fait que la majeure partie des tests utilisés dans ces études ont été développés sur la base d'un seul antigène du SARS-CoV-2, ce qui peut affecter la performance de ces tests. En fait, dans de nombreux rapports, les anticorps anti-SARS-CoV-2 sont connus pour réagir de manière croisée avec des antigènes d'autres agents pathogènes tels que le virus de la Dengue et Plasmodium. Dans notre étude, nous avons comparé deux tests sérologiques : un test ELISA et un test rapide, tous deux basés sur la protéine Spike du SARS-CoV-2, et la prévalence déterminée à l'aide des mêmes échantillons était de 36 % et 30 %, respectivement, montrant une mauvaise concordance entre les tests et illustrant à quel point il peut être difficile d'interpréter l'analyse sérologique dans le contexte africain, où plusieurs autres agents pathogènes sont endémiques. Afin d'avoir un test sérologique d'une grande précision, nous avons développé un test multiplex lors du premier confinement national en France qui s'est déroulé du 17 mars au 11 mai 2020. Nous avons utilisé des échantillons de plasma prélevés sur des patients confirmés COVID-19 hospitalisés au Centre Hospitalier Universitaire (CHU) de Montpellier. Après avoir évalué notre test, nous avons trouvé une précision de 100 % (IC à 95 % : 100-100) pour la protéine Spike et de 99,9 % (IC à 95 % : 99,7-100) pour la nucléoprotéine. Après avoir défini les valeurs seuils pour les deux antigènes, nous avons considéré comme positif tout plasma qui réagissait simultanément aux deux antigènes testés. En effet, ce critère permettait de déterminer une séroprévalence proche de la vraie diffusion du SARS-CoV-2 à Kinshasa car il réduirait la surestimation due aux réactions croisées. De plus, des études réalisées après la deuxième, troisième et quatrième vague de COVID-19 ont également révélé que la séroprévalence

avait augmenté d'un facteur de deux à trois par rapport à celle documentée après la première vague, dépassant 50 % au Mali, au Cameroun et au Zimbabwe.

Nos résultats, ainsi que ceux d'autres rapports sur la séroprévalence du SARS-CoV-2 dans les pays africains, nous ont permis de tirer deux conclusions importantes :

(1) Le SARS-CoV-2 s'est largement propagé en Afrique après la première vague de la pandémie et encore plus après les vagues suivantes atteignant plus de 50 % dans plusieurs pays, en dépit des mesures de confinement prises par les gouvernements de ces pays. Force est donc de constater que les données communiquées par les instances officielles sont largement sous-estimées et une étude évalue le facteur moyen de sous-estimation à 8,5. En effet, cette sous-estimation révèle également les limites des systèmes de santé dans plusieurs pays africains, dont la RDC. Ces limitations font référence à la faible capacité de diagnostic, au manque de structures d'hospitalisation appropriées et à la faiblesse de la surveillance au niveau communautaire. En RDC, le taux de dépistage quotidien moyen par RT-PCR du SARS-CoV-2 est de 0,01/1000 habitants, allant de 30 à 5000 tests par jour pour une population de plus de 90 millions d'individus, avec une positivité moyenne de 7,86 % prenant en compte la période du 11 mars 2020 au 23 septembre 2022.

(2) Bien que le nombre de cas et de décès signalés de COVID-19 aient été sous-estimés, nous pensons que le nombre de formes graves de COVID-19 et le nombre de décès étaient inférieurs à ceux signalés dans d'autres régions du monde telles que l'Amérique et l'Europe. Contrairement aux autres continents, l'Afrique a très rarement rapporté des scénarios de surpopulation hospitalière ou de décès simultanés de plusieurs patients. Nous pensons que la pyramide des âges en Afrique, qui se distingue par une densité relativement élevée d'individus jeunes < 40 ans (seulement 3% à 6% ≥ 60 ans), peut expliquer la différence de présentation clinique de la pandémie entre l'Afrique et d'autres continents. L'âge a été reconnu comme un facteur important lié à la gravité de l'état de la COVID-19. De plus, la forme sévère de COVID-19 a une forte corrélation avec les troubles métaboliques comme le diabète et l'obésité ainsi que les maladies cardiovasculaires qui sont également significativement associées à l'âge. Ces études n'ont pas pu montrer que d'autres facteurs, tels que les conditions climatiques et une potentielle immunité partielle préexistante due à l'exposition à d'autres coronavirus, pourraient avoir une influence significative sur la faible

gravité de la maladie COVID-19 en Afrique. Ces résultats permettent aux pays à ressources limitées, comme les pays africains, qui ont eu du mal à obtenir du matériel et des vaccins pour lutter contre la pandémie à prendre des mesures efficaces et efficientes basée sur des évidences pour lutter contre la pandémie.

Ce travail, réalisé dans le cadre de notre projet de thèse, a montré que des patients infectés par EBOV peuvent développer une forme paucisymptomatique et parfois ne pas être diagnostiqués par RT-PCR, peut-être parce qu'ils ont été testés après la période virémique ou qu'ils ont une faible charge virale. Nous avons également noté que les nouveaux traitements spécifiques utilisés lors de la 10^{ème} épidémie d'Ebola en RDC semblent être associés à la baisse rapide des anticorps au fil du temps, bien que nous ne puissions pas exclure l'influence d'autres facteurs. Nos travaux sur le SARS-CoV-2 ont montré que le virus s'est largement propagé à Kinshasa, contrairement aux données officiellement publiées. La fréquence très élevée des épidémies d'Ebola et la survenue de la pandémie de SARS-CoV-2 démontrent donc la nécessité de poursuivre les recherches sur l'interface homme-animal et d'approfondir la compréhension de la réponse immunitaire humaine contre ces pathogènes sur les moyens de prévention tels que les vaccins et les différents traitements déjà développés et en cours de développement.

General Introduction

Emerging Infectious Diseases (EIDs) are defined by Stephen Morse as “infections that are newly recognized in a population or have existed but are rapidly increasing in incidence or geographic range” (Morse, 1995). EIDs include such as previously unrecognized diseases like human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), severe acute respiratory syndrome (SARS), Ebola hemorrhagic fever, and Nipah virus encephalitis (The National Academies, 2009). Researchers have identified and classified pathogens that cause disease in human. Indeed, 1,407 of approximately identified pathogens are classified as viruses, bacteria, parasites, fungi, and helminths of medical interest (Woolhouse *et al.*, 2005). Of these, around 15% are associated with disease emergence and/or reemergence (Sabin *et al.*, 2020). Based on the Center for Disease Control and Prevention (CDC) report, around three out of four (75%) of them have a zoonotic origin and most of them are viruses (CDC, 2022d).

Pathogens have emerged for centuries and have caused multiple outbreaks and pandemics that have resulted in millions of deaths and disabilities around the world. The 20th century has remarkably recorded dramatical pandemics such as the 1918-1920 influenza pandemic that caused at least 50 million of deaths (CDC, 2019), acquired immune deficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) firstly identified in 1981, and spreading quickly worldwide causing a global number of deaths estimate to be more than 40 million, ranged between 1.8 and 2.3 million every year (Bongaarts *et al.*, 2009). In 1976, two non-related deadly hemorrhagic fever outbreaks were recorded consecutively in Sudan and in the Democratic Republic of the Congo (DRC). Later, the virus of concern was identified as Ebola virus genus which belongs to the *Filovirus* family (Bowen *et al.*, 1977; Bres, 1978). In the present century, the global world has been facing several infectious threats, and some of them have caused outbreaks and global pandemic such as the severe acute respiratory syndrome coronavirus (SARS-CoV) that started in China (2003) (WHO, 2022c), the 2012 middle east coronavirus (MERS-CoV) in Middle east and South Korea (WHO, 2022b), and the current pandemic of severe acute respiratory syndrome coronavirus two (SARS-CoV-2) (Hasoksuz *et al.*, 2020).

Indeed, there is no need to demonstrate that emerging diseases play a non-negligible role in the deterioration of the public health status worldwide. Therefore, early detection, treatment of patients and, if necessary, quarantine of patients are public health tools to combat them. Over the past decades, medicine has made several scientific advances, particularly in the field of infectious disease diagnosis ([The National Academies, 2009](#)). This includes, for example, the development of molecular methods such as polymerase chain reaction (PCR) and sequencing, which have improved sensitivity and, above all, specificity in detecting microorganisms. Fighting virus epidemics, in fact, means, in particular, being able to make an inventory for each pathogen distribution and evaluate the effectiveness of the treatments that are being evaluated or that have already been validated by the authorities in charge. Bearing in mind that the human immune system is believed to produce the specific immune response to each infectious agent, the detection and evaluation of the components of the immune response could be used to determine the global prevalence of the virus, its dynamic, and the degree of protection among populations at risk of being infected.

The detection of antibodies and their characterization by serological methods allows a better understanding of emerging diseases and their dynamics in order to better adapt infection control measures to each specific pathogen ([Metcalf *et al.*, 2016](#); [Ohst *et al.*, 2018](#)). For example, Ebola virus is known to cause very severe and fatal clinical infection and may result in very few possible cases of asymptomatic patients compared to infection caused by SARS-CoV-2. In addition, several candidate vaccines against the Ebola virus and have been evaluated in the population in recent years. Therefore, the detection of antibodies and their characterization (frequency, neutralization efficiency and duration) using serological tests, which have a longer diagnostic window in detecting current and past infections, can play an important role in better understanding the dynamics and distribution of Ebola virus and SARS-CoV-2 in population of interest.

In the present thesis project, we aimed to continue research on EIDs in the DRC; in order to understand the distribution of Ebola virus in suspected cases diagnosed as negative Ebola virus disease (EVD) cases. Also, we aimed to understand the longitudinal evolution of anti-Ebola virus antibody in EVD survivors that were treated with specific antiviral and monoclonal antibodies. Finally, we looked for the seroprevalence distribution of the newly discovered SARS-CoV-2 infection in an African setting.

Bibliographic Synthesis

Chapter 1. Insight viral emerging infectious diseases

1.1. Background

Emerging Infectious Diseases (EIDs) are infections that are newly recognized in a population that cause a public health concern (WHO, 1997). Reemerging infectious diseases are known diseases that reappear and are rapidly increasing in incidence or geographic range but they were or were not considered in the past as a public health threat (Sabin *et al.*, 2020; WHO, 1997). Thus, two factors are important for a pathogen to be considered as emerging: the rapid increase of the incidence and/or spread in a larger or new geographic range.

It is estimated that more than 6 out of every 10 known infectious diseases are transmitted to human from animals, and today 3 out of every 4 new emerging infectious disease in humans are of zoonotic origin (CDC, 2022d). In the last two decades, emerging infections with viruses were the major causes of epidemics and pandemics recorded worldwide causing at least 10 million of deaths per year, mainly in tropical countries (Bhadoria *et al.*, 2021; Fenollar *et al.*, 2018). Furthermore, EIDs have a high burden on public health but also on global economies (Fenollar *et al.*, 2018).

The present chapter will give an update on the evolution and the main factors leading to the emergence of viral infectious pathogens. It will describe public health measures and tools used to contain their spread with a focus on the emergence of Ebolavirus (EBOV) and the Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2).

1.2. Steps required for an infection to emerge

For the majority of scientists, the interaction between infectious agents, their hosts, and the environment are the elements of the triad for EIDs (Figure 1) (Morens *et al.*, 2020). Globally, the pathogen emergence process has been conceptualized using two sequential levels. First, the introduction and adaptation of a pathogen into a naïve new human population: after infecting a new host population, the pathogen may have an overall reproductive number of less than one ($R_0 < 1$) leading to his extinction, but some may evolve and increase their virulence to give $R_0 > 1$ which represents the scenario that the pathogen

may persist and spread into a new host with a risk for the development of an epidemic. The second level is the sustained spread of the pathogen into the new human population without the involvement of the original reservoir (R. Antia *et al.*, 2003; Slingenbergh *et al.*, 2004; The National Academies, 2009).

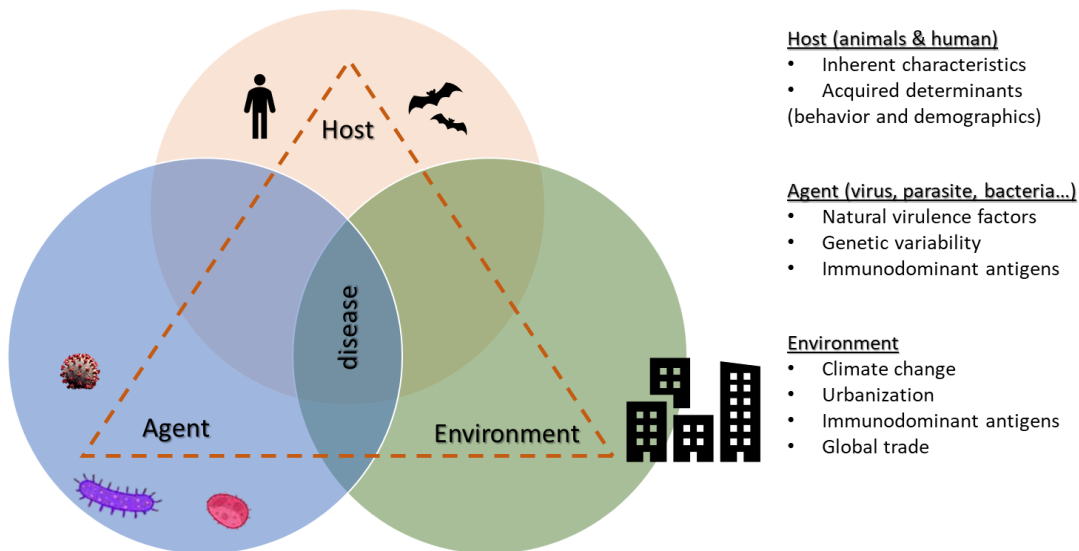


Figure 1. Component of the emerging infectious triad and their determinants.

The process that result in viral pathogen emergence includes a triad of human and animal hosts, the viral agent, and the environment. These emerging components are determined by their respective enhancement factors. Adapted from (Morens *et al.*, 2020)

In detail, the ability of zoonotic diseases to spread among humans is classified through 5 stages (Wolfe *et al.*, 2007):

- 1) spread only in animals: the pathogen can circulate only in animals but cannot cross to humans under natural conditions. Are excluded all artificial transmission pathways such as blood transfusion and organ transplant process;
- 2) primary infection: the pathogen can only infect humans from animals under natural conditions. However, the primary infection does not result in secondary cases;
- 3) limited epidemic: the pathogen can be transmitted from animals to humans, resulting in few secondary human cases. High-fatality zoonotic pathogens, such as the Ebola virus or the Marburg virus, remained classified as stage 3 for several years of their evolution before progressing to the higher stage;

4) long outbreak: the pathogen is transmitted from animals or humans with many transmission cycles. There are both sylvatic cycle and a relatively long and sustain sequence of secondary infection in humans;

5) exclusively human pathogen: The pathogen is only transmitted to humans. In fact, this can happen through two mechanisms; First, the pathogen evolved from an ancestral pathogen that was present in both human and animal species phylogenetically close to humans. Second, an animal pathogen might have colonized humans more recently and evolved into a specialized human pathogen.

Several factors may be involved in the control of transitions between emergences stages. Among them are described, the probability-per-unit-time (p) between the new host and the existing host which seems to play a key role in transition between stage 1 to stage 2. In fact, the susceptibility of a new host to a specific agent may increase with the increase of the existing host population. However, p may decrease with the increase in phylogenetic difference between the new host and the existing one. Passage of an emerging pathogen from stage 2 to stage 3 or 4 mostly depends on the animal and human hosts behavior. Human population size and transmission efficiency constitute barriers that avoid pathogens to evaluate to stage 5 of containment in sustained human transmission (Figure 2).

Effectively, the interaction between hosts (humans and animals), infectious agents, and the environment plays a key role in the emergence of pathogens that impact public health and its socioeconomic determinants (Sabin *et al.*, 2020).

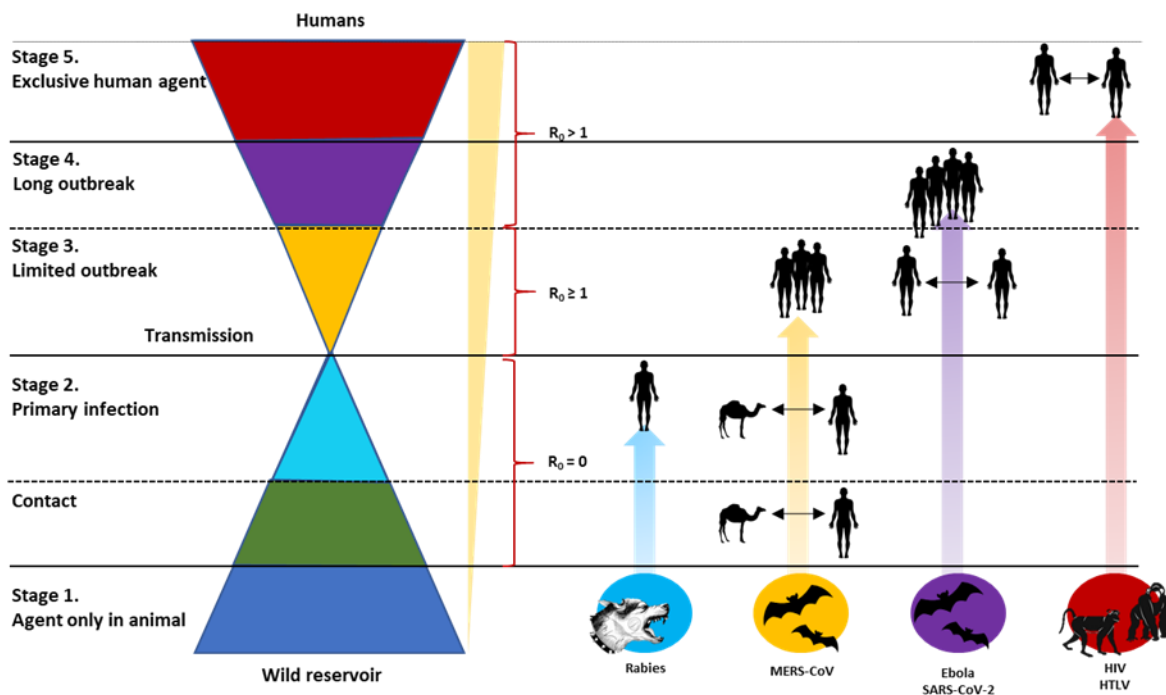


Figure 2. Emergence of viruses from animal reservoir and probability of transmission.

The emergence of viral pathogens is a multi-step process, which mainly involves the viral transmission to humans through various possible contacts with infected body fluids (initial transmission) and its subsequent spread in the human population. In the case of the Ebola virus, for example, the virus generally emerged from its natural reservoir, which would be the fruit bat, and then transmitted to humans directly or via the intermediate host. The host's immune and viral genetic traits determine the adaptation of the virus to its new host. The basic reproductive rate also determines the ability of a newly introduced virus to survive in a population. Adapted from (Gessain *et al.*, 2013; Jill-Léa Ramassamy, 2021; Wolfe *et al.*, 2007).

1.3. Factors affecting the emergence of infectious diseases

Factors associated with emergence of infectious diseases have to be in synergy in order to create conditions that are favorable for the emergence of EIDs. For example, the interrelation between climate change, globalization, close contact between humans and animals, urbanization, and human-environment interface has significantly contributed to the acceleration of EIDs emergence frequency in the 21st century (El Amri *et al.*, 2020). Based on the biogeographical analysis, Peterson suggested a framework to explain the spatial distribution of infectious diseases. Indeed, the framework includes the dispersal capacity of the pathogen in combination with the abiotic environment such as climate and biotic environment such as habitat which determine the ability of the pathogen or disease to realize

its full geographic potential (Peterson, 2008) while Lambin *et al.* have emphasized the role of the spatial and temporal interaction between agents, their habitat and susceptible human hosts (Lambin *et al.*, 2010). Therefore, physical geography, environment, reservoir hosts, vectors, pathogens, human factors, management, and disease distributions are interdependent elements that can influence both the real and observed distribution of specific pathogens in space and through time (Murray *et al.*, 2018).

Successively, we will describe the role of the pathogen, the host, and the environment in the process of emergence.

1.3.1. Pathogen agent's role in the emergence of infectious diseases

The infectious agent is the main component of the triad responsible for the emergence of infectious diseases and this largely depends on its natural or acquired intrinsic virulence characteristics (Morens *et al.*, 2020).

1.3.1.1. Genetic variability

Changes in many ecological niches make it difficult for many microorganisms to adapt and cross species. However, some infectious agents have unstable genetic material and can therefore adapt to changes in their environment (Morens *et al.*, 2020). Ribonucleic acid (RNA) viruses, e.g., filoviruses, coronaviruses, and flaviviruses have deficient or absent polymerase error correction mechanisms, and this may result in a multiplicity of viral variants. Combined with other factors, the genetic diversity or plasticity of certain infectious agents may play a key role in the occurrence of cross-species infections (Asjo *et al.*, 2006).

During their lifecycle, viruses are commonly transmitted within their host reservoir and can often spill-over to humans when opportunities occur but usually fail to sustain transmission between humans, this was called “viral chatter” phenomenon or “local epidemic”. (Wolfe *et al.*, 2005). Consequently, the factors that promote evolution from the primary infection to local epidemics are poorly understood. However, the widely accepted mechanism for the emergence of a new pathogen appears to begin with the high level of viral chatter, which increases the diversity of viruses and strains that can be transmitted to humans, increasing the likelihood of transmission of pathogens that replicate successfully, and ultimately

increase the likelihood of transmission of human-adapted viruses ([Apetrei et al., 2004](#); [Epstein, 2004](#)).

Changes in the genomes of both RNA and Deoxyribonucleic acid (DNA) viruses as described above can occur through mutations, genetic drift, genetic shift, reassortment, and recombination. These changes may result in silent mutations or may produce proteins that allow the virus to adapt to a new environment ([Asjo et al., 2006](#)). In addition to the genetic diversity, cell tropism, ability to escape host innate immune system, and antigenic immunodominance are other factors that are linked to the emergence of infectious agents ([Morens et al., 2020](#)).

1.3.2. The place of the host in the emergence of infectious diseases

There are individual or collective characteristics that may underlie the development of infectious diseases in humans. The human organism contains cells that express receptors or have cell surface properties necessary for viral entry and replication ([Morens et al., 2020](#)). Moreover, the immune status of individuals or the entire population may contribute to the emergence of infectious diseases ([Choi, 2021](#)).

1.3.2.1. Cell, tissue, and host tropism

The ability of the virus to adapt to a new host, to replicate and to spread is determined by the host cell surface receptors, generally known as “viral tropism” ([Susi, 2021](#)). Viral tropism can be subdivided into three levels depending on the histological structure of the human host that is receptive to the virus. Thereby, we call “cell tropism” when the virus replicates in only one cell type, “tissue tropism” when the virus replicates in a particular tissue or organ but not in another, and “host tropism” is determined by the limit of the virus replication in a specific host ([McFadden et al., 2009](#); [Neal, 2014](#)).

Mechanisms of viral entry into cells are extremely complex and variable:

(1) some viruses enter cells using binding to two different proximate receptors, a primary and secondary receptor, e.g. many *flaviviruses* and *lentiviruses* require different receptors for binding and fusion ([Morens et al., 2020](#));

(2) many other viruses can use more than one molecule as a receptor, which complicates the viral tropism pattern, and can lead to systemic clinical infection, e.g. *measles virus* (Lyons *et al.*, 2015; Neal, 2014).

This definition of viral tropism, however, can only be applied to viruses with lytic replication cycles that produce virions. In the case of a latent infection-induced virus, the virus can successfully enter cells and maintain the ability to produce latent infection while it cannot undergo productive replication (McFadden *et al.*, 2009).

1.3.2.2. Host immunity

Humans have a well-structured immune system acting as a natural barrier against pathogens (Choi, 2021). In this architecture, the innate immune system is the first line defense and plays an important role in anti-microbial immunity and associated pathologies. Innate immune response involves multiple changes in molecules and cells in response to a viral infection. For example, type I and III interferons (IFNs) are rapidly released after viral recognition by the host innate system. These cytokines are potent multifunctional and play an important role in the early defense by blocking viral replication by the mechanism called “antiviral state”, and further by recruiting multiple immune cells to the site of viral infection (Morens *et al.*, 2020; Woodland *et al.*, 2008).

The adaptive viral immune response is more specific, and the magnitude and quality of the response increases as the infection progresses. Adaptive immune responses are antibody and cellular mediated, and involve an interdependence between T and B cells underlying the innate response relationship (Morens *et al.*, 2020; Woodland *et al.*, 2008). Adaptive immune system also consists in a large amount of memory B cells that are able to produce antibody in case of any reinfection in the future by the same pathogen. These antibodies can also provide cross protection against pathogens that are close to the pathogen that was responsible of the previous infection.

When an outbreak starts in a population, a possible pre-existing partial immunity of a subset of the population against the pathogen causing the outbreak can interfere with the early viral spread and possibly the evolution of the outbreak (Morens *et al.*, 2020). Protection can also be provided by pre-existing antibodies which can cross-protect against the newly introduced

pathogen that is antigenically close to the previously induced immune response. For example, the reported incidence of monkeypox in humans has apparently increased after the cessation of smallpox vaccination following its eradication, suggesting a possible cross-protection between smallpox-induced antibodies against monkeypox virus (Lloyd-Smith, 2013). Finally, there is evidence that severity of infectious diseases and susceptibility of the population to specific pathogens may be related to host genetic variables associated with the innate immune response (Morens *et al.*, 2020).

1.3.2.3. Human activities and behavior

Human lifestyle and behavior can influence emergence of pathogens. Activities such as urbanization, globalization, tourism, trade, and international transport may increase the risk of displacement and introduction of pathogen agents in a new naïve host population resulting in a possible infectious emergence (El Amri *et al.*, 2020; McArthur, 2019). The centralization of food production chains using intensive agriculture, and new technological tools can create optimal conditions for the emergence of diseases. In addition, human behavior plays also a key role in the infectious emergence process; e.g., international travel/trade, crowding, sex behavior can imbalance the environmental order and finally change the ecologic niches (El Amri *et al.*, 2020; Morens *et al.*, 2020).

1.3.3. The role of the environment in the emergence

The environment has a major impact on the process of the infectious agent emergence (Slingenbergh *et al.*, 2004). Climate change and global warming have been recognized for a long time as the major factors that are linked to risk of infectious diseases emergence. The 21st century will record the highest temperature as the Intergovernmental panel on climate change (IPCC) projected. There will be an increase in temperature of 0.2°C ($\pm 0.1^\circ\text{C}$) per decade with an expected mean temperature increase between 1.4°C and 4.4°C by the end of the century, and that will lead to an increase of sea levels (Rupasinghe *et al.*, 2022).

Climate change, and other factors such as urbanization, land-use change, international travel, and socio-economic drivers (poverty, lack of clean water and sanitation, ...) have synergetic effects on changes of the epidemiological characteristics of infectious agents, e.g., there is an association between urbanization, climate change, and the emergence of

arboviruses (Dengue, Yellow fever, Zika, ...) that are transmitted by mosquitos of the genus *Aedes*. Indeed, urbanization increases the larval development rate, the adult lifespan, the capacity of the vector, and finally the likelihood of transmitting the disease by *Ae. albopictus* (Li *et al.*, 2014; Zahouli *et al.*, 2017).

Tropical and equatorial forests are known to serve as hotspots for microorganisms. The number of microbial, animal and plant species increase from the Arctic to the equator, suggesting a great diversity and concentration of microorganisms in the intertropical regions which corresponds to the large number and diversity of animals. Encompassing much of the Congo Basin, the DRC is home to a vast equatorial forest, second only to the Amazon rainforest (Encyclopedia Britannica, 2022). Therefore, the complexity of microorganism species as well as the diversity of animals acting as hosts may play an important role in the emergence of infectious diseases in humans. For example, of the 1200 known bat species, each bat species harbors approximately 17-18 viruses that can cause moderate to severe infections in humans (Guégan *et al.*, 2019). Furthermore, the role of the DRC in the emergence of infectious diseases has recently been highlighted by the spread of *Monkeypox virus*, which is highly endemic in the DRC but has now spread to other countries causing human infections with a different clinical expression than previously described (Bunge *et al.*, 2022).

Thus, the described triad of pathogens, host and environment and related risk factors should be deeply studied in order to elucidate the current knowledge on EIDs and to fill the gaps on what is currently not yet understood.

1.4. Implication in public health response

The public health burden of infectious diseases has been significant from ancient times to the present day, while global health has improved over the past 30 years. In fact, 25% of the approximately 60 million deaths worldwide each year are caused by infectious diseases, which can also affect the quality of life of those who are treated. In 2019, for example, six infectious diseases were among the top ten causes of disability-adjusted life years (DALYs) in children under the age of ten (Nii-Trebi, 2017; Vos *et al.*, 2020). Additionally, people with infectious diseases may experience mental, psychological and emotional problems

with difficulty of reintegrating into their communities, for example, Ebola virus disease (EVD) survivors and people with leprosy feel ashamed or feel rejected by their community (Delamou *et al.*, 2017; Nii-Trebi, 2017). After all, EIDs cost developing countries billions of dollars every year and increase adult poverty, which can also impact children's education. (Nii-Trebi, 2017).

EIDs involve people, animals, the environment and ecological factors that act interdependently, making it sometimes difficult to achieve the objectives of control measures. Indeed, the first line of defense against emerging pathogens is their rapid detection and identification, leading to the rapid implementation of preventive measures that can prove highly effective in combating outbreaks of new diseases (The National Academies, 2009). However, early recognition of a new pathogen can be a challenge, as it will likely depend primarily on clinical signs, such as reporting groups of cases with unusual symptoms. Therefore, identifying and stopping emergence pathogen at the source requires increased collaboration and investment to bring together experts from multidisciplinary areas involving human, animal and socioeconomic science disciplines such as clinicians, laboratory, epidemiologists, veterinarians, anthropologists, and ecologists. This approach is summarized by the concept “One-health” (McCloskey *et al.*, 2014).

A one-health concept is defined as “a collaborative, multisectoral, and transdisciplinary approach - working at the local, regional, national, and global levels - with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment” (CDC, 2022c; McCloskey *et al.*, 2014; Sinclair, 2019). One-health issues include zoonotic diseases, antimicrobial resistance, food safety, vector-borne diseases, environmental pollution and other threats to human, animal and environmental health (CDC, 2022c). Thus, governments, international agencies, and global organizations are collaborating to implement the aims of One-health (McCloskey *et al.*, 2014). Also, strengthening one-health partnerships globally could lead to the development of mechanisms to rapidly detect and respond to pandemics in animals and humans around the world. The knowledge gained by this approach can make it possible to change health policy in order to improve the protection of human and animal health (McCloskey *et al.*, 2014).

Chapter 2. Ebola virus and Ebola virus disease

2.1. Ebolavirus

2.1.1. Ebolavirus discovery

Between June and November 1976, an hemorrhagic fever epidemic of unknown cause was reported in the areas of Nzara, Maridi, and Lirangu in Sudan (Currently South Sudan) (WHO/International Study Team, 1978a). In the same period, between 1 September and 24 October 1976, the Ministry of Health of Zaïre (Currently the Democratic Republic of the Congo (DRC) reported an outbreak with similar symptoms in a Catholic Mission in Yambuku in the Bumba health zone (HZ) (WHO/International Study Team, 1978c). The World Health Organization (WHO) and the international community were requested to assist with the identification of etiology of this outbreak. Based on symptoms, the first hypothesis was that it could have been Yellow fever, Lassa fever, Congo-Crimean hemorrhagic fever, or Marburg disease, as they were previously identified in Africa (Bres, 1978). Further analysis done in three laboratories (Porton Down in the United Kingdom, CDC in the United States of America, and the Prince Leopold Institute of Tropical Medicine in Antwerp, Belgium) on the samples from Sudan and Zaïre have shown that the virus resembled to Marburg virus but with antigenic differences observed by indirect immunofluorescence (I.F.A). Thus, the new virus belonged the *Filovirus* family and was named Ebola in reference of the small river in Yambuku/Zaïre (Figure 3) (Bowen *et al.*, 1977; Bres, 1978).

In terms of symptoms reported in patients from both outbreaks, the patients with the disease in Zaire had fewer respiratory symptoms, a shorter clinical course and a higher mortality rate than those in Sudan, while surviving patients appeared to have cross-reacting antibodies to Sudanese and Zairian strains apart from neutralization tests that later distinguished between the two serotypes (WHO/International Study Team, 1978a, 1978c). However, scientists later showed that the two outbreaks were caused by two different strains of the Ebola virus: the *Zaire Ebolavirus* and the *Sudan Ebolavirus*, and this discovery led to the conclusion that the virus came from two different sources and spread to humans independently in each of the affected areas (CDC, 2022b; McCormick *et al.*, 1983).



Figure 3. Ebola river near Yambuku, where the first Ebola Virus Disease outbreak was reported. (<https://robertjprince.net/2014/10/14/ebola-poor-black-mans-african-epidemic/>)

2.1.2. Classification

The classification of Marburg and Ebola viruses has changed over the years since their discovery in 1967 and 1976, respectively, and updates have been proposed to the International Committee on Taxonomy of Viruses (ICTV) for validation. *Marburgvirus* and *Ebolavirus* belong to the order of *Mononegavirales* introduced in 1991 as a taxon “to embrace families of viruses with similar genomic organization and replicative strategies” (Pringle, 1991, 1997). In the *Mononegavirales* family, viruses are enveloped and have a linear single-stranded RNA genome. In general the gene is characterized by 3′-UTR-core proteins genes-envelope protein genes-polymerase gene-5′-UTR (Kuhn *et al.*, 2010). Based on the accepted classification carried out during the ratification vote in October 2018, the *Mononegavirales* Order includes eight families: *Artoviridae*, *Bornaviridae*, *Filoviridae*, *Lispiviridae*, *Myonaviridae*, *Nyamiviridae*, *Paramyxoviridae* (Amarasinghe *et al.*, 2019). Thus, *Ebolavirus* and *Marburgvirus* are part of the *Filoviridae* family.

Filoviruses exhibit a unique filamentous morphology, and family members are characterized by properties of mononegaviruses plus other common characteristics such as

give rise to a viral hemorrhagic fever in (certain) primates and humans; infecting primates, porcids (pigs) or chiroptera (bats) in nature; having long (≈ 19 kb) genomes, a characteristic they only share with henipaviruses and “jeilongviruses” (other mononegaviruses: ≈ 8 – 16 kb); having a genomic RNA that encodes seven structural proteins in the order 3′-UTR-NP-VP35-VP40-GP-VP30-VP24-L-5′-UTR, one of which (VP24) is unique to family members, and one of which (VP30) is partially analogous to a protein expressed only by pneumoviruses, etc. (Kuhn *et al.*, 2010). Genera *Dianlovirus*, *Thamnovirus*, *Cuevavirus*, *Ebolavirus* and *Marburgvirus* are family members of *Filoviridae* family (Figure 4) (Amarasinghe *et al.*, 2019). We will describe more in detail the six lineages of the genus *Ebolavirus* (Ebola virus) (Table 1)

Table 1. Current taxonomy of the *Mononegavirales* family *Filoviridae*.

Genus name	Species name	Virus name	Virus name abbreviation
<i>Cuevavirus</i>	<i>Lloviu cuevavirus</i>	Lloviu virus	LLOV
<i>Dianlovirus</i>	<i>Mengla dianlovirus</i>	Mengla virus	MLAV
<i>Ebolavirus</i>	<i>Bombali ebolavirus</i>	Bombali virus	BOMV
	<i>Bundibugyo ebolavirus</i>	Bundibugyo virus	BDBV
	<i>Reston ebolavirus</i>	Reston virus	RESTV
	<i>Sudan ebolavirus</i>	Sudan virus	SUDV
	<i>Tai Forest ebolavirus</i>	Tai Forest virus	TAFV
	<i>Zaire ebolavirus</i>	Ebola virus	EBOV
<i>Marburgvirus</i>	<i>Marburg marburgvirus</i>	Marburg virus	MARV
		Ravn virus	RAVV
<i>Striavirus</i>	<i>Xilang striavirus</i>	Xilang virus	XILV
<i>Thamnovirus</i>	<i>Hangjiao thamnovirus</i>	Huang virus	HUJV

Source (Kuhn 2019)

Zaire ebolavirus (ZEBOV), today known as the *Ebolavirus* (EBOV) since the recent classification, has the highest average EVD fatality rate around 50%, ranging from 25% to 90% (WHO, 2021). The first outbreak of EBOV was reported in Yambuku/Zaire in 1976, and 318 cases were recorded with 280 deaths (88%) (CDC, 2022b), and EBOV was also responsible of the West African Ebola outbreak in 2014 (Baize *et al.*, 2014). EBOV strains

are documented in outbreak in DRC, Gabon, and the Republic of the Congo; the strains have a genome with two or three gene overlaps (VP35/VP40, GP/VP30, VP24/L; VP24 and L overlap only if the second, rather than the first, transcription termination signal of VP24 is used); and they have a full-length genomic sequence with <30% differences from the suggested type virus which is *Zaire ebolavirus* (Kuhn *et al.*, 2010).

Sudan ebolavirus (SUDV). The virus emerged in 1976 in Nzara, Sudan where the first outbreak caused 284 cases with 151 deaths (53% of case-fatality rate) (CDC, 2022b). SUDV strains were involved in the other outbreaks reported from Sudan in 1977 and in 2004 (CDC, 2022b) and in the outbreaks reported in Uganda successively in 2000, 2011 and 2012. Strains members of SUDV lineage are characterized by having common properties of Ebola virus genus plus: being reported in the Republic of Sudan and the Republic of Uganda; having genomes with three gene overlaps (VP35/VP40, GP/VP30, VP24/L); having a full-length genomic sequence with differences from the type virus which is SUDV by <30% (Kuhn *et al.*, 2010).

Reston ebolavirus (RESTV). This strain of the Ebola virus was identified in Virginia/United States America (USA) during an outbreak of simian hemorrhagic fever virus (SHFV) in crab-eating macaques from Hazleton Laboratories (now Labcorp Drug Development) in 1989 (Jahrling *et al.*, 1990). The virus has also been implicated in the outbreak in non-human primates in Pennsylvania, Texas and Siena, Italy. In both cases, the affected animals were imported from a facility in the Philippines (Jahrling *et al.*, 1990). The members of the *Reston ebolavirus* strain are characterized by having the properties of ebolaviruses with in addition: being reported in south-east Asia (pigs in China, and in the Philippines; having genomes with two gene overlaps (VP35/VP40, VP24/L); display a full-length genomic sequence with differences from the type virus of the lineage which is RESTV by <30% (Kuhn *et al.*, 2010). RESTV infects several species of monkeys, pigs, ferrets, bats and either naturally and/or experimentally. After experimental infection, immunosuppressed rodents, monkeys and ferrets and STAT-1 knockout mice have developed severe disease with lethal consequences, whereas immunocompetent rodents usually did not (Haddock *et al.*, 2021).

Tai Forest ebolavirus (TAFV), also known as "Côte d'Ivoire ebolavirus". The virus was discovered among chimpanzees from the Tai Forest in Côte d'Ivoire, in 1994 (CDC, 2022b). The meat of infected western red colobus monkeys (*Procolobus badius*) was suspected as the source of the virus, although there is no formal evidence for this (Formenty *et al.*, 1999). A human case with the *Tai Forest ebolavirus* has been reported in a primatologist performing autopsies on dead chimpanzees. The strains of *Tai Forest ebolavirus* are characterized by having the properties of Ebola virus and: being reported in Côte d'Ivoire; having genomes with three gene overlaps (VP35/VP40, GP/VP30, VP24/L); having a full-length genomic sequence different from Ebola virus genus type by $\geq 30\%$ but must have difference with the type virus of the TAFV strain $< 30\%$ (Kuhn *et al.*, 2010).

Bundibugyo ebolavirus (BDBV). The virus was reported on November 24, 2007 in Bundibugyo district in Uganda. After confirming samples tested by US National Reference Laboratories and the CDC, WHO confirmed the presence of the new strain of Ebola virus. A total of 149 cases have been reported and 37 deaths (24.8% mortality rate). Another outbreak caused by the Ebola Bundibugyo virus was recorded in the east of DRC in 2012, close to border with Uganda (CDC, 2022b). The members of the strains *Bundibugyo ebolavirus* share the common characteristics with other Ebola viruses, and express other features such as: being responsible of outbreaks in Uganda and in DRC; having genomes with three gene overlaps (VP35/VP40, GP/VP30, VP24/L); having a full-length genomic sequence displaying less 30% of difference with the BDBV type virus (Kuhn *et al.*, 2010).

Bombali ebolavirus (BOMV). The new strain of *Ebolavirus* was described recently by researchers who were doing a survey to identify the natural reservoir of *Ebolavirus* as well as other filoviruses that could circulate in wildlife in Sierra Leone, West Africa. Among 1278 samples collected from 535 animals (244 bats, 46 rodents, 240 dogs, 5 cats), four bats (three Little free-tailed bats (*Chaerephon pumilus*) and one Angolan free-tailed bat (*Mops condylurus*)) were positive using a broadly reactive conventional polymerase chain reaction (cPCR) assay at *Filovirus* family level but their genomes showed 75% nucleotide identity to other ebolaviruses with no evidence of recombination (Goldstein *et al.*, 2018). Thus, the newly discovered strain was named *Bombali ebolavirus* based on the geographical location of its detection. (Goldstein *et al.*, 2018). The *Bombali ebolavirus* was also detected in organs

and excreta of an Angolan free-tailed bat in Kenya in 2018 (Forbes *et al.*, 2019), and in *Mops condylurus* bats in Guinea between 2018 and 2019 (Karan *et al.*, 2019).

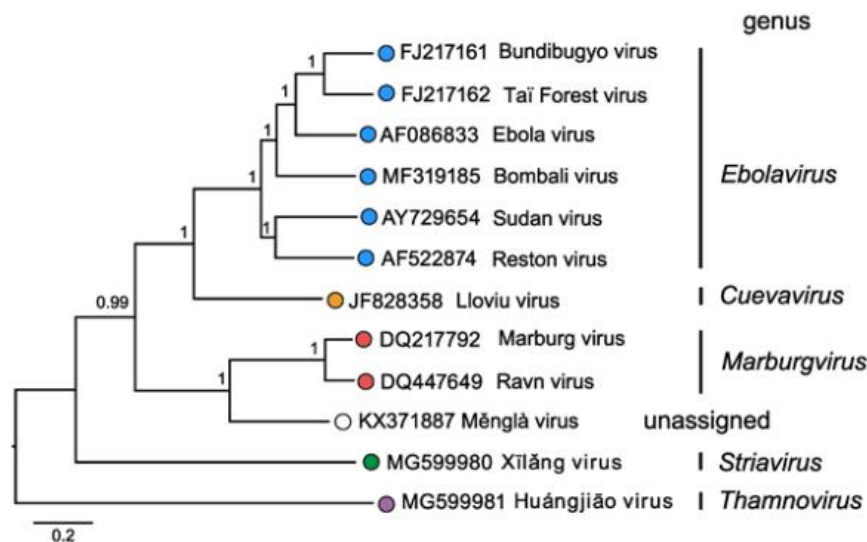


Figure 4. Phylogeny of the Filovirus genomic sequences.

This is a maximum-likelihood tree (midpoint-rooted) inferred by using coding-complete or complete filovirus genomes demonstrates the six distinct clades (genera) of *Filoviridae* family. Sequences were aligned using Clustal-Omega version 1.2.1 (<http://www.clustal.org/omega/>) and were manually curated in Geneious version R9 (<http://www.geneious.com>). Trees were derived in Fast Tree Version 2.1 using a General Time Reversible (GTR) model with 20 gamma rate categories, 5,000 bootstrap replicates and exhaustive search parameters (-slow) and pseudo-counts (-pseudo). The numbers near the nodes on the trees indicate the bootstrap values in decimal form. The branches of the tree are scaled to nucleotide substitutions per site. Branch tips indicate GenBank accession numbers (Kuhn 2019).

2.1.3. Structure of Ebolavirus

The morphological structure of *Ebolavirus* was first described by electron microscopic observation after cell culture in 1977 using human specimens from the outbreaks in Zaire and Sudan (Bowen *et al.*, 1977; Johnson *et al.*, 1977). The mature form of the *Ebolavirus* particle exhibits long filamentous rods with a uniform diameter of ~ 80 nm and an average length of ~ 1,250 nm while his peak infectivity is associated with virions ~ 805 nm in length (Hoenen *et al.*, 2006). Globally, the virions of filovirus show pleomorphism which includes long filaments (Latin-filum means thread), shaped like a “6”, a “U”, or a “circle” (Singh *et*

al., 2017). However, EBOV particles appear longer and predominantly filamentous compared to other strains of Ebola virus, e.g., SUDV lineage seems to overproduce particles with unusual shapes, particles with conglomerate envelopes, or naked nuclei. This could explain why SUDV is less virulent than the EBOV strain when comparing the mortality rate during the outbreaks caused by EBOV or SUDV, although nothing is yet clear about it (Kuhn, 2008). Despite morphological differences, the genomes of Ebola virus genus are closely related. Grard et al. suggested that the genetic diversity among Ebola virus species ranges from 25% to 35% (Grard *et al.*, 2011), while Kuhn et al. clarified in the proposed revised taxonomy of the family *Filoviridae* that the species to be included in the Ebola virus genus have $\geq 30\%$ of genetic diversity, but the difference within the same lineage or strain must be $< 30\%$ (Kuhn *et al.*, 2010). Singh et al. have reported observed molecular divergences in Ebola virus genus. For example, strains of TAFV and BDBV are less diverse with 17.8% mean genetic diversity between sequenced strains. The mean genetic diversity of EBOV was 26.1% and 26.8% in comparison with TAFV and BDBV, respectively. Similarly, RESTV strains show 36.3%, 37.4%, and 37.6% of mean genetic diversity compared to EBOV, TAFV, and BDBV, respectively (Singh *et al.*, 2017).

The EBOV genome is an enveloped, non-segmented, negative single-stranded RNA with a size of 18,890 bases. The genome encodes seven structural proteins and one nonstructural protein (nsp) in the following order: 3'-leader→nucleoprotein (NP) gene→viral protein (VP) 35 gene→VP40 gene→glycoprotein (GP) gene→VP30 gene→VP24 gene→polymerase (L) gene→5'-trailer. The conserved leader (30 non-coding regions) and the trailer (50 non-coding regions) carry genomic replication promoters and packing signals. Viral particles have a central core known as the ribonucleo-protein (RNP) complex called also “nucleocapsid”, that consists of NP, VP35, VP30, L and the viral RNA. This RNP complex is surrounded by a lipid envelope, with which the remaining proteins GP1,2, VP40 and VP24 are associated; these three proteins function as surface glycoprotein, major matrix protein and minor matrix protein respectively (Figure 5). These viral antigens play distinct roles in virus replication and pathogenesis (Baseler *et al.*, 2017; Hoenen *et al.*, 2006; M. N. James, Edward, J.D. (editors),, 2017). Furthermore, each EBOV gene is delimited by 3' and 5' untranslated regions (UTRs) that contain conserved transcription initiation and termination signals. Although most genes are separated by intergenic regions of different lengths, some genes overlap in portions of the UTR. All genes except GP, which encode a

total of three glycoproteins, are monocistronic. The main product of GP is soluble GP (sGP). By processing RNA by the viral polymerase complex, Ebolavirus regulates transmembrane GP expression and produces a second poorly soluble glycoprotein (ssGP). This RNA processing is a distinctive feature between Ebola and Marburg viruses (Baseler *et al.*, 2017).

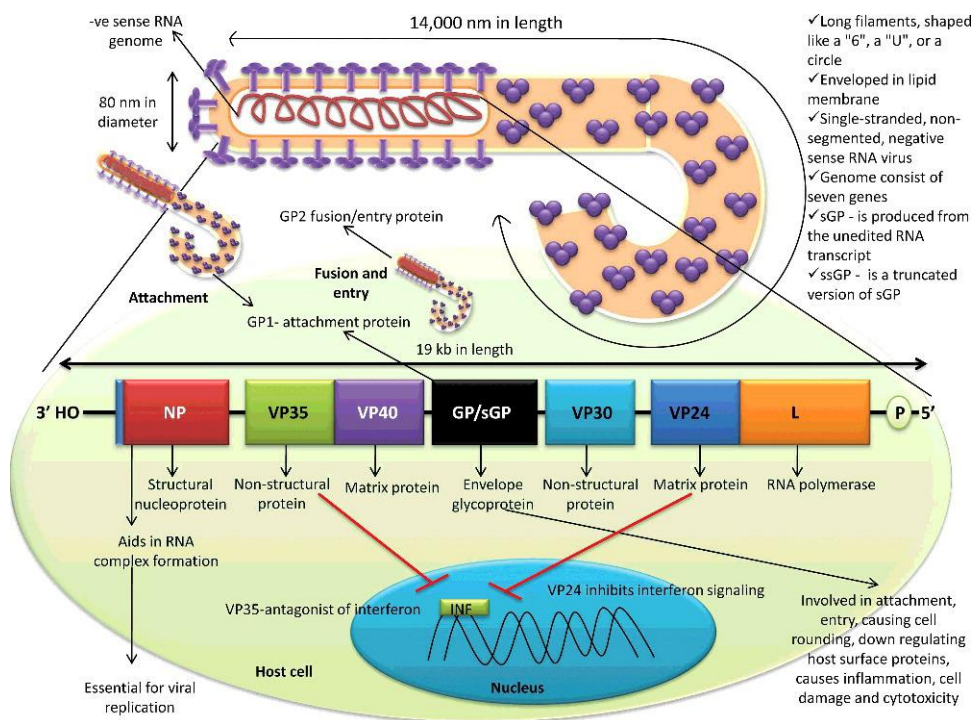


Figure 5. Structure of the genus Ebola virus and its genome.

Ebola virus possesses negative-sense RNA genome with exceptionally 14000 nm length with 3' nucleoprotein and 5' RNA polymerase end (Singh *et al.* 2017).

2.1.4. Ebolavirus lifecycle

Although significant progress has been made in understanding the mechanisms of the EBOV life cycle in host cells, many aspects remain poorly understood. EBOV can infect a large range of mammalian cells, such as monocytes, macrophages, and dendritic cells, and this function makes it difficult to identify the cellular proteins needed for the viral attachment on the cell surface (Singh *et al.*, 2017; D. S. Yu *et al.*, 2017). The present part of the chapter 2 will describe the cell lifecycle of EBOV step by step (Figure 6).

Attachment, uptake, viral entry. The transmembrane protein glycoprotein (GP) facilitates cellular attachment and entry and is extensively glycosylated, particularly in the glycan-rich mucin-like domain. GP is formed of three heterodimers consisting of subunits GP₁ and GP₂ that are connected by a disulfide bond (Ghosh *et al.*, 2021). Attachment to target cells is mediated by binding of the GP₁ transmembrane viral envelope to cell surface factor (s). GP₁ contains three distinct domains: receptor binding domain (RBD); glycan cap; and a heavily O-linked glycosylated mucin-like domain (MLD). RBD interacts with cellular receptors, and the glycan cap interacts with the internal fusion loop of GP₂, which is essential for GP₂-mediated membrane fusion, preventing premature fusion (Kuhn *et al.*, 2006; D. S. Yu *et al.*, 2017). Moreover, EBOV cell surface interactions differ from other interactions described with the cell surface enveloped virus / receptor because EBOV GP amino acid residues do not interact with cell surface receptors. In contrast, these viruses bind to target cells through two relatively non-specific receptor types: C-type lectins (CLECs), which interacts with EBOV GP glycans, and the PtdSer receptor, which interacts with the PtdSer viral envelope (Moller-Tank *et al.*, 2015). However, the mechanism that lead to virion internalization through binding of CLECs to N- and O-linked glycans on EBOV GP is poorly understood but there is evidence that cell lacking CLEC expression remain permissive for EBOV infection, suggesting that CLEC-independent uptake mechanism also occur (Moller-Tank *et al.*, 2014; G. Simmons *et al.*, 2003).

The mechanism that triggers EBOV uptake is still unknown. Otherwise, the uptake of EBOV into the host cell involves various endocytic pathways. Several studies have shown that macropinocytosis and other factors on the host cell and virus particle size are important for the virus entry (Moller-Tank *et al.*, 2015). First, the endocytic pathway of EBOV entry appears to be dependent on the endocytic enzymes cathepsin B/L and cholesterol, which are the major component of caveolae and lipid-rafts, and EBOV GP-transduction can be activated by the implication of several Guanosine Triphosphate-ases (GTPases) such as RhoB, Rac 1, and CDC42 (Bavari *et al.*, 2002). Second, identification of the indirect role of low pH in EBOV-GP mediated membrane fusion. Acidic conditions affect cathepsin activity, which affects GP-mediated fusion for number of enveloped viruses such as EBOV or Marburg (Cleavage of GP) (Bavari *et al.*, 2002). Third, fusion induced by GP cleavage by cathepsin activity after viral internalization in the endosome is independent of pH, suggesting that there are unidentified cellular factors important for EBOV entry that

are sensitive even at low pH. The cleaved GP interacts with the host receptor NPC1 in order to trigger the fusion of the viral and endolysosomal membranes (Falzarano *et al.*, 2007; Hoenen *et al.*, 2019). Currently, few factors controlling the migration of endosomal virus have been described, e.g., the two-pore channel (TCP2) may play an important role in the viral entry process, but its precise role is controversial (Sakurai *et al.*, 2015; J. A. Simmons *et al.*, 2016). The fusion of viral and endolysosomal membranes allows the release of the nucleocapsid complex into the host cell cytoplasm, after which the ribonucleo-protein (RNP) complex associated with the VP24 protein will undergo dissociation allowing the relaxation of rigid nucleocapsids into transcription and replication-competent RNP complex structures (Banadyga *et al.*, 2017; Watt *et al.*, 2014).

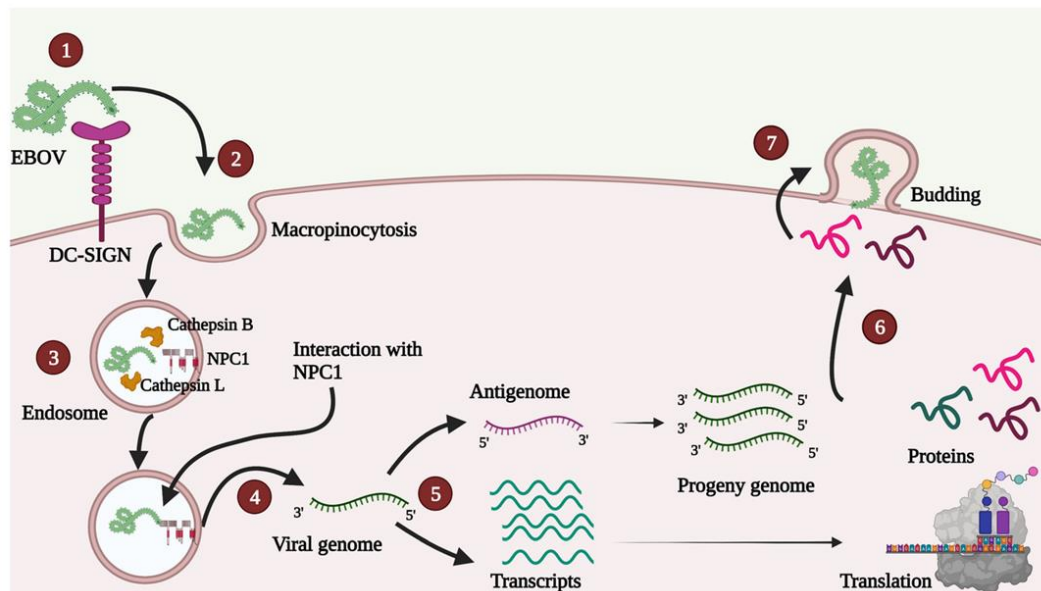


Figure 6. A simple diagrammatic representation of various steps in the EBOV life cycle.

1. Binding or attachment: EBOV can interact with essential various host cell receptors for binding. The figure shows a DC-SIGN receiver as an example. 2. Uptake: it occurs primarily by micropinocytosis as noted, although other processes such as clathrin-mediated endocytosis and caveolin-mediated endocytosis are also contemplated. 3. Entry-GP1 proteolysis in the endosome allows for viral interaction with the host receptor cholesterol transporter Niemann-Pick C1 (NPC1; shown in red). 4. Release: After membrane fusion, the viral genome is released into the host cell's cytoplasm. 5. Transcription and Replication: Primary transcription occurs in the cytoplasm of the host cell followed by translation. The antigenome is used as a template for the synthesis of progeny genomes. 6. Transport: various proteins are transported near the plasma membrane 7. Assembly and budding: VP40 plays a crucial role in the assembly, formation and budding of virus-like particles (VLPs) (Sahil Jain *et al.* 2021).

Transcription, replication, assembly, and release. Upon entry into the cytoplasm and membrane fusion, RNP is released from the virion and serves as a template. Primary transcription of the viral genome occurs in the cytoplasm of the host cell, resulting in the production of the first wave of all viral mRNAs, which are translated and complement the components of the RNP complex proteins, and then additional secondary transcriptions are produced (Hoenen *et al.*, 2019; D. S. Yu *et al.*, 2017). The replication process begins first with the synthesis of complementary positive-stranded RNA (cRNA) which is produced as RNP and secondly, the released RNP generates viral genomic RNA which will be packaged into the virion. Furthermore, the formation and fusion of RNP is an important step for the transcription, replication and assembly of negative ssRNA because the complete replication cycle of ssRNA negative viruses such as EBOV is present only in the form of RNP which always acts as a RNA template for synthesis and assembly of the virion (Hoenen *et al.*, 2019; D. S. Yu *et al.*, 2017).

The EBOV polymerase L synthesizes, by transcription, the mRNA coding for GP₁, GP₂ associated with the virion, and two other soluble glycoproteins, including sGP and ssGP, then allows their production (Mehedi *et al.*, 2011; Volchkov *et al.*, 1995). In addition to this, host factors play also a role in replication and transcription of the EBOV. Among these factors, DNA topoisomerase 1 (TOP1), a nuclear protein that binds to the helical structures of double-stranded DNA to unwind the helices for transcription and replication, can interact directly with the viral polymerase and with the structure of the Viral RNA. Another host factor that supports transcription and replication is the RNA binding protein Staufen 1 (STAU1) which plays a critical role in EBOV replication by coordinating the interactions between the viral genome and the RNA synthesis machinery (Fang *et al.*, 2018; Takahashi *et al.*, 2013). Finally, VP24, the late phase of RNA synthesis, allows condensation of the RNP complex in quiescent replication and transcription while packaging competent nucleocapsids through its physical association with the RNP complex (Banadyga *et al.*, 2017; Hoenen *et al.*, 2019).

The newly synthesized nucleocapsids will be transported to the surface of the host cells in an actin-dependent pathway along with the matrix protein VP40. However, the viral glycoprotein is transported to the cell surface via the secretory pathway, where it is modified post-translationally by the addition of *O*- and *N*-linked glycans, as well as by furin-mediated

cleavage in mature GP₁ and GP₂ subunits (Hoenen *et al.*, 2019; Volchkov *et al.*, 1998). After all viral proteins are synthesized, virion assembly will occur at the plasma membrane through VP40 activity involving the endosomal sorting complex require for transport (ESCRT) which is a host factor that help for the production of multivesicular bodies (Hoenen *et al.*, 2019). Next, the virion is released by budding. The process is initiated by the interaction between VP40 and the host's inner membrane. VP40 has an electrostatic and the hydrophobic components that are associated with the plasma membrane phosphatidylserine (PS). VP40 undergoes oligomerization on the inner leaflet of the plasma membrane triggered by PS-containing membranes with normally affinity (Adu-Gyamfi *et al.*, 2012). In addition, the GP₂ and the tetherin act as another major pathway in EBOV virion budding. Tetherin is an IFN- α induced, cell-surface-protein-based tether that induces the retention of the virus in the cell membrane while GP₂ acts as a tetherin antagonist through its hydrophobic membrane-spanning domain (MSD) (Lopez *et al.*, 2010; Neil *et al.*, 2008; Vande Burgt *et al.*, 2015).

2.1.5. Ebolavirus pathogenesis

In recent years, significant progress has been made in understanding the pathogenic mechanisms underlying Ebola virus disease (EVD). Data from EVD in humans and animal models (immune competent animals: mouse, rhesus monkey, guinea pig) have led to better knowledge of the pathogenesis of EBOV. Although most human cells are permissive to EBOV, mononuclear phagocytes (macrophages, monocytes, Kupffer cells and microglia) and dendritic cells (DC) are the main targets of EBOV (Bray *et al.*, 2005; Geisbert, Hensley, *et al.*, 2003; Geisbert *et al.*, 1992; Geisbert *et al.*, 2015; Schnittler *et al.*, 1998; Takada *et al.*, 1997). EBOV infection in mononuclear cells and DCs partially damages their functions by suppression of innate immune responses, including type I interferon responses, allowing them to further initiate inflammation and coagulation, but they cannot prevent systemic spread of the virus. Therefore, cytokines released by infected cells recruit additional target cells to sites of infection, resulting in virus spread to resident macrophages and DCs in body tissues, leading to massive release of pro-inflammatory mediators and substances vasoactive (Bray *et al.*, 2005; Mahanty *et al.*, 2004). However, impaired coagulation does not appear to be a direct effect of endothelial cell infection, but may be due to overexpression of tissue factor in mononuclear phagocytes, leading to overactivation of the

extrinsic coagulation pathway, followed by consumption coagulopathy and finally disseminated intravascular coagulation (Figure 7) (Geisbert, Young, Jahrling, Davis, Kagan, *et al.*, 2003; Geisbert, Young, Jahrling, Davis, Larsen, *et al.*, 2003).

Replication in infected cells is very efficient, resulting in rapid, high-peak viremia and necrosis throughout the process, which may involve toxic effects of viral glycoproteins and other structural proteins or matrix proteins. Likewise, the severity of the infection may also be explained by the fact that EBOV infection impairs the development of antigen-specific immune responses, in part by preventing dendritic cells from activating T cells. (Bray *et al.*, 2005; Mahanty *et al.*, 2004). Lymphocytes and natural killer (NK) cells are known to be not infected by EBOV leading to lymphopenia observed in EVD patients over the course of the clinical disease (Bray *et al.*, 2005). Therefore, the mechanism that lead to the induction of the lymphocyte apoptosis is poorly understood, but several virus-infected macrophages induced mediators, including tumor necrosis factor alpha (TNF- α), Fas and its ligand, TNF- α -related apoptosis-inducing ligand (TRAIL) and Nitric oxide (NO), seem to be involved in inducing lymphocyte apoptosis (Hensley *et al.*, 2002; Reed *et al.*, 2004). Consequently, the early loss of the lymphocytes in EVD may contribute to the acceleration of viral replication and bodily dissemination by the lack of interferon gamma (IFN- γ) that activates macrophages and other inflammatory cells involved in restriction of the viral replication (Bray *et al.*, 2005). On the other hand, the loss of CD4 lymphocytes may explain the absence of EBOV specific IgM and IgG in patients who died from EVD (Baize *et al.*, 1999; McElroy *et al.*, 2018; Rivera *et al.*, 2016).

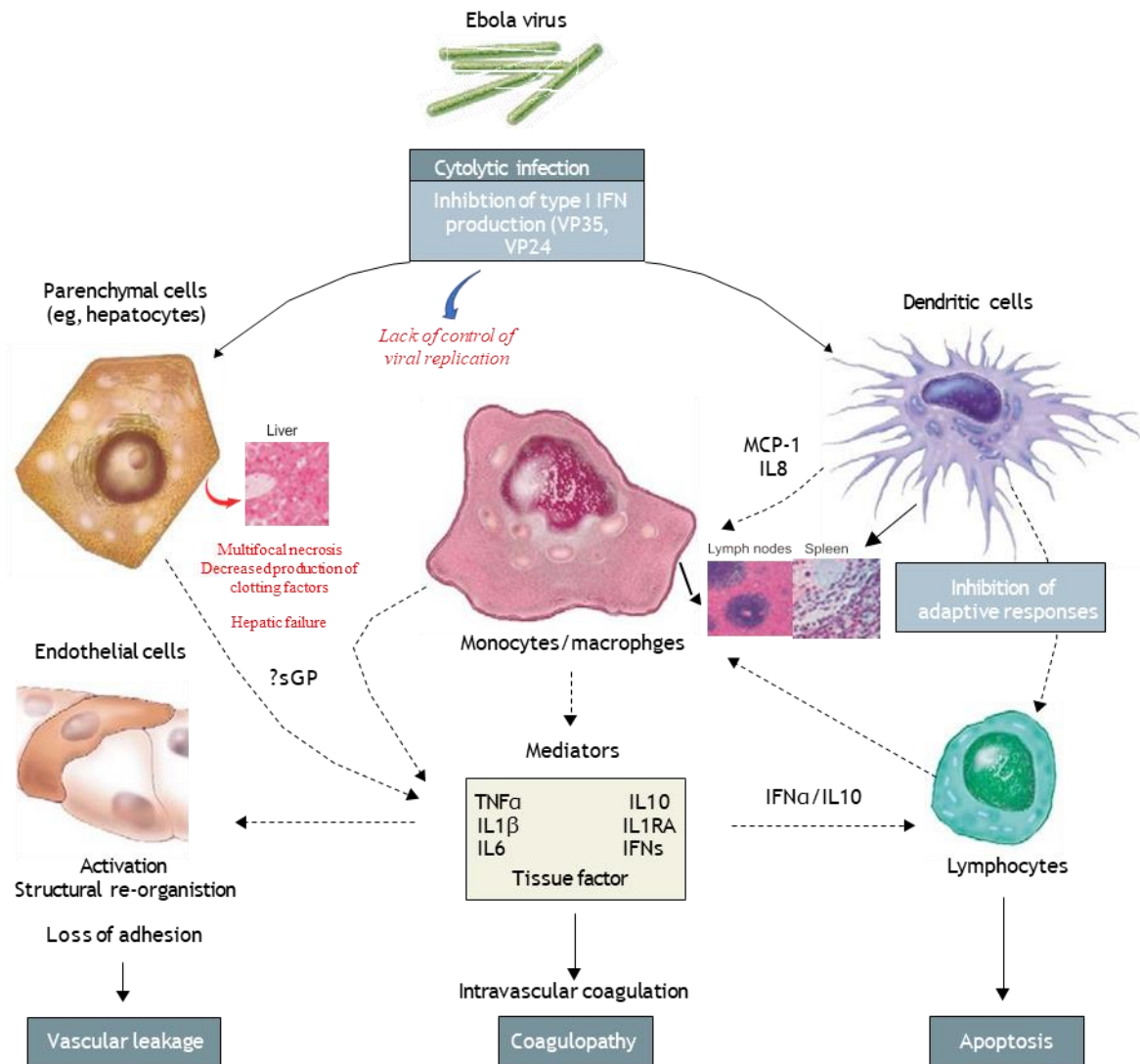


Figure 7. Pathogenesis of Ebola virus induced clinical disease. Adapted from (Siddhartha M. *et al.*, 2004).

2.1.6. Reservoir and Ecology

During 40 years, many researchers studied thousands of animals, insects and plants to try to find the natural reservoir of the Ebola viruses, but it is still unknown. However, the geographical location of Ebola virus transmission indicates that since the virus was discovered in Sudan and DRC in 1976, and because most outbreaks have been reported in Central Africa, suggest that the virus's natural reservoirs can be found most likely in tropical forests of this continent (Baseler *et al.*, 2017; CDC, 2022b). Although gorillas, chimpanzees, antelopes or other and other mammals may be involved in transmitting the virus to humans, they do not survive and are not considered natural reservoirs of the

Ebolavirus. In general, reservoir animals may not suffer from acute disease, despite the presence of the virus in their organs, tissues and blood. Viruses can thus be transmitted from host to host or through an intermediate host or vector and persist in the environment (CDC, 2022b). We will examine here the main animals' species supposed to be involved in the emergence of EVD. These include bats, non-human primates and other animals.

2.1.6.1. The role of bats in the cycle of Ebola virus

Since the emergence of the Ebola virus in 1976, several researchers believe that bats could be natural reservoirs of the Ebola virus as bats are increasingly being involved in zoonotic virus emergence. Humans can become infected through intermediate or amplifying hosts such as antelopes and non-human primates, or by handling and eating infected bushmeat, but also indirectly by consuming fruit contaminated by biological excreta of Ebola-infected bats, such as saliva, urine or feces. To test the hypothesis, Swanepoel et al. have experimentally infected plants and animals with Ebola virus, and they have observed that frugivorous and insectivorous bats were able to support viral replication, infection, and survived to the infection (Swanepoel et al., 1996). However, only one study reported EBOV RNA-positive samples collected in frugivorous bats in Gabon and the Republic of Congo between 2001 and 2005. In that report, a total of 13 liver and spleen samples from three frugivorous bat species were positive for EBOV RNA by PCR. Among them 4/21 were *Hypsignathus monstrosus*, 5/117 were *Epomops franqueti*, and 4/141 were *Myonycteris torquata* (Leroy et al., 2005). Ebola virus was confirmed in 7 samples by sequence analysis. Further research is thus needed to confirm these findings as no other study has yet detected EBOV RNA in frugivorous bats till now. Nevertheless, other *Filoviridae* viruses have been reported bats in recent years. For example, the newly described Ebola virus strain named *Ebola bombali* was isolated in insectivorous bats, *Chaerephon pumilus* and *Mops condylurus* (Goldstein et al., 2018). *Cuevavirus* RNA was isolated in European bats (Negredo et al., 2011), and several studies have amplification and sequencing results of Marburgvirus from bats samples (Jones et al., 2015; Kuzmin et al., 2010; Swanepoel et al., 2007; Towner et al., 2009; Towner et al., 2007).

Serological tests were applied to animal samples to understand Ebola virus dynamics but also to estimate the seroprevalence of Ebola virus specific antibodies in African bats to fill

the gap of the lack of molecular evidences. Several techniques have been used to assess the seropositivity in animal samples to EBOV antigens such as Enzyme Linked Immunosorbent Assay (ELISA), or the multiplex immunoassay technics (Luminex). Leroy et al. have detected and reported 16 bats sera positive to anti-Immunoglobulin G specific for Ebola virus in a group of three bat species (Leroy et al., 2005). In a large serosurvey of Ebola virus in frugivorous and insectivorous bats conducted in three countries: Guinea, Cameroon, and the DRC, the researchers analyzed 4,022 blood samples of more than 12 frugivorous and 27 insectivorous species bats and they reported a range of seropositivity to EBOV antigens from 0.05% to 0.92% while a range of 0% to 0.75% was reported for seropositivity to SUDV antigens (De Nys et al., 2018). Antibodies were detected in at least 7 frugivorous bat species. In addition, Lacroix et al. have assessed the presence of antibodies in bats during the 2018 EBOV outbreak in Equateur and East provinces in DRC. Using multiplex detection tools, they reported an overall seroprevalence ranging from 1.2% to 11.4% for reactivity to at least one antigen among 925 samples analyzed. They did not detect viral RNA in the 676 bats samples they analyzed (Lacroix et al., 2021).

It is becoming more and more evident that it is very difficult to carry out studies in wild animals to identify the reservoir of a virus, in particular that of the Ebola virus. The type of samples to be taken, but also sampling precautions to protect animal life and operator are some of the limits of these studies, but also the very high number of species and colonies of bats can considerably reduce the probability of detection and above all isolating Ebola virus from them. However, the fact that Ebola antibodies have been detected in bats in places where no case of Ebola-related illness has ever been reported suggests that bats may play an important role in the origin of EVD (De Nys et al., 2018; Lacroix et al., 2021). Furthermore, bats have been documented as the source of the 2007 EVD outbreak in Luebo, in DRC where the putative index case bought freshly killed bats from hunters for consumption (Leroy et al., 2009). Although epidemiological investigations during the multicountry 2014-2016 EVD outbreak in West Africa did not found molecular evidence for the zoonotic origin of the EBOV virus, they have documented exposure of the local population in Guinea to frugivorous and insectivorous bats (Mari Saez et al., 2015). Ultimately, urbanization growth, invasion of forested areas, and close interaction with wildlife animals, are the factors that can be associated with the higher frequency of EVD outbreaks in the last decade (Sivanandy et al., 2022). Therefore, we have to continue exploration and research on bats

and other wildlife animals to increase the probability of discovering the natural EBOV reservoir.

2.1.6.2. The role of non-human primates in the emergence of Ebola virus

Non-human primates have been associated with filoviruses for many years, suggesting that they may be natural reservoirs of filoviruses. For example, Marburg virus disease has been reported in German laboratory workers exposed to grivets (*Chlorocebus aethiops*) imported from Uganda. These non-human primates have been considered as a source of infection, while the mechanism by which grivets may have become infected has been poorly understood (Ristanovic *et al.*, 2020). Reston ebolavirus which was imported from the Philippines was the identified aetiology of a simian hemorrhagic fever virus outbreak in Virginia and Pennsylvania in USA in 1989 (Jahrling *et al.*, 1990). Another evidence of filovirus infection in non-human primates was recorded in 1994 in Ivory Coast where a primatologist who did autopsies on dead chimpanzees was infected by TAFV (Kuhn *et al.*, 2010). During three Ebola outbreaks in Gabon between 1994 and 1996, chimpanzees and gorilla were also reported dead in the forest and were detected to be infected with Ebola virus. All initial patients in the spring 1996 Ebola outbreak in Gabon shared the experience of butchering carcasses of chimpanzees (Leroy, Rouquet, *et al.*, 2004). From 2002 to 2003, an estimated 5,500 endangered western gorillas were killed by Ebola virus at the Lossi Sactuary in the Republic of Congo (Bermejo *et al.*, 2006). Indeed, natural reservoirs are animals that are susceptible to EBOV infection but do not develop disease or die from it. However, several studies have reported that non-human primates can be infected by Ebola virus under natural conditions or during experimental laboratory infection, and they are susceptible to develop clinical illness with high mortality rate suggesting that they could act as amplifier or intermediate host for the Ebola virus between humans and bats that are likely supposed to act as natural Ebola virus reservoirs (Corti *et al.*, 2016; Geisbert, Hensley, *et al.*, 2003). In addition to that, Ayouba *et al.* have assessed seropositivity to Ebola virus antigens in 36 different species of monkeys and apes (2,322 and 2,327 respectively) from Cameroon, DRC and Ivory Coast. Indeed, using the simultaneous presence of antibodies to both glycoprotein and nucleoprotein as positivity criteria, only one individual was declared as positive to Ebola virus antigens, suggesting that it likely developed anterior Ebola infection (A. Ayouba *et al.*, 2019). In a study conducted in Kenya using indirect

immunofluorescence, Johnson et al. reported 1.6% (3/184) of Ebola virus seroprevalence in baboons that were in captivity. In a multicountry serosurvey conducted in wild animals as explained above, Leroy et al. analyzed samples by ELISA and reported 11% of seropositivity to Ebola virus antigen in chimpanzees, 5.7% in gorillas, 4% in baboons, 1.8% in mandrills, and 0.9% in cercopithecines. The variability of these results could be explained by the technique used which can be sensitive to unspecific reactions (Leroy, Telfer, *et al.*, 2004). These findings emphasize the hypothesis that non-human primates may act as intermediate hosts in Ebola virus emergence but do not act as reservoirs.

2.1.6.3. The role of rodents, pigs, and other mammals in Ebola virus emergence

Tropical forests host several species of rodents known to be carriers of multiple pathogens and a source of protein for local communities. In the search for the reservoir of the Ebola virus disease, several authors have been particularly interested in the investigation of African rodents to evaluate their role in the emergence of Ebola virus. The results of a wildlife survey conducted in Cameroon and DRC, where 651 rodent blood samples were collected showed all samples negative for viral RNA but also seronegative to Ebola virus antigens (Breman *et al.*, 1999). In a zoonotic investigation during the 2017 Ebola virus outbreak in Likati in DRC, authors trapped 476 small mammals such as rodents, shrews, and bats but also got samples from 11 hunted mammals and tested for Ebola virus RNA and for detection of antibodies. Indeed, they have reported all samples to be negative for antibodies against Ebola virus (Gryseels *et al.*, 2020). Contrariwise, a study conducted in a population with no history of Ebola outbreaks in DRC reported a significant association between Ebola virus seropositivity and people who were in contact with rodents (Mulangu *et al.*, 2018). These results should be interpreted with caution, as the serological technique used may induce non-specific reactions and, consequently, an overestimation of reported Ebola virus seroprevalence. Indeed, it is very preferable to use multiplex analysis or strict positivity criteria to minimize the non-specific antigen-antibody response.

Since an outbreak of RESTV occurred in pigs in the Philippines, where six individuals working on pig farms, as well as those working with pig products, have developed antibodies (IgG) against RESTV (Barrette *et al.*, 2009), researchers have increasingly studied the role of pigs in the emergence of the Ebola virus. Although RESTV did not lead

to clinical disease in humans, the outbreak resulted in the culling of more than 6,000 pigs in measures taken to stop the spread of the virus (Mogato, 2009). Indeed, in order to understand interaction between pigs and EBOV, experimental studies have been conducted on pigs and have shown that pigs are susceptible to EBOV (Kobinger *et al.*, 2011), with high viral replication resulting in very high viral titers. The pig appears to develop EBOV respiratory disease with diffuse and fatal lung involvement. Nasal, oral and rectal secretions contain viral RNA and represent contaminants. Naïve pigs were contaminated by infected pigs and developed the disease. Experimentally infected pigs have also been shown to transmit the infection to non-human primates (Kobinger *et al.*, 2011; Weingartl *et al.*, 2012). Furthermore, serological assessment of natural EBOV exposure in pigs has been reported by Fischer *et al.* in Sierra Leone in 2018, and in Guinea in 2019 after the West Africa outbreak of EBOV in humans. Three out of 400 porcine serum samples reacted with EBOV NP antigen but did not neutralize the virus in Sierra Leone (Fischer *et al.*, 2018) while the study from Guinea that included 308 porcine serum samples reported 6.2% (19/308) and 4.2% (13/308) of seropositivity to EBOV NP using ELISA and Western blot respectively. The study reported also 4 samples that reacted to SUDV GP (Fischer *et al.*, 2020). More recently, a study conducted in Uganda on 658 pig samples between December 2015 and October 2016, 46 (7%) were seropositive in ELISA; 27 to SUDV NP, 8 to EBOV NP, and 11 to both EBOV and SUDV NP (Atherstone *et al.*, 2021). Indeed, these results show that pigs are most likely also exposed to the Ebola virus and therefore pig farming, which is increasingly practiced in Africa, must be monitored by human and animal public health measures to avoid a possible amplification of the Ebola virus in pigs.

In this context, it is becoming increasingly clear that mammals, but also domestic animals, could play a role in the transmission of the Ebola virus. Antibodies to the EBOV strain have been detected in dogs studied in Liberia and Gabon (Allela *et al.*, 2005; Haun *et al.*, 2019). In 2001, during the Ebola epidemic in Gabon, seropositivity to EBOV antigens, in particular glycoprotein, was correlated with the regions affected by the epidemic (Allela *et al.*, 2005). In fact, most of the seroprevalence studies cited used the ELISA technique, based on whole viral lysate, and therefore these results should be interpreted with caution due to their low specificity.

2.2. Ebolavirus disease

2.2.1. History of epidemics

Since Ebola was discovered in 1976 when two consecutive outbreaks of fatal hemorrhagic fever occurred in Sudan and in the DRC, EBOV has emerged periodically and infected people in several African countries. Most outbreaks are attributable to independent single EBOV spillover introductions into the human population from an unknown animal reservoir by unknown way of cross-species transmission (CDC, 2022b; Jacob *et al.*, 2020). To date, a total of 45 EVD local and imported outbreaks have been recorded worldwide. Of these, 41 have been reported in Africa, and 15 in the DRC (Table 2; Figure 8) (CDC, 2022b), but the virus has probably existed before any recorded outbreaks according to the viral and epidemiological available data. For example, molecular analysis of the 2014 EBOV in West Africa showed that the lineages responsible for the outbreaks diverged from a common ancestor around 2004, suggesting that each outbreak represents an independent zoonotic event from the same genetically viral population diversified in its natural reservoir (Gire *et al.*, 2014). Additionally, Carroll *et al.* Reported 2% diversities among sequence viruses collected in human samples during several recorded outbreaks in the Democratic Republic of the Congo. Using Bayesian coalescence analyzes, they estimate that the most recent common ancestor of these strains of EBOV (Luebo 2008) occurred 48 years earlier, or around 1960 (Carroll *et al.*, 2013). Furthermore, four of the six known strains of the *Ebolavirus* genus lead to human disease-associated infections (EBOV, SUDV, BDBV, TAFV), but only three of them (EBOV, SUDV, BDBV) have been associated with Ebola virus outbreaks while RESTV can infect human but does not causes clinical illness (Barrette *et al.*, 2009), and there is no human infection recorded yet in relation with BOMV (Jacob *et al.*, 2020). Whereas, the majority of these epidemics occurred in remote rural areas, in recent years, outbreaks reached also urban and semi-urban areas, e.g., during the largest EVD outbreak reported in Guinea, Liberia, and Sierra Leone from 2014 to 2016 the virus spread also in urban regions of the three countries with 28,610 confirmed cases, and 11,308 (39%) deaths (Breman *et al.*, 2016; CDC, 2022b; Na *et al.*, 2015). Likewise, the EVD outbreak reported in 2018 in eastern DRC (North and South Kivu and Ituri provinces), which caused 3,470 confirmed cases and 2,287 (66%) deaths, also took place in semi-urban areas of these provinces (CDC, 2022b).

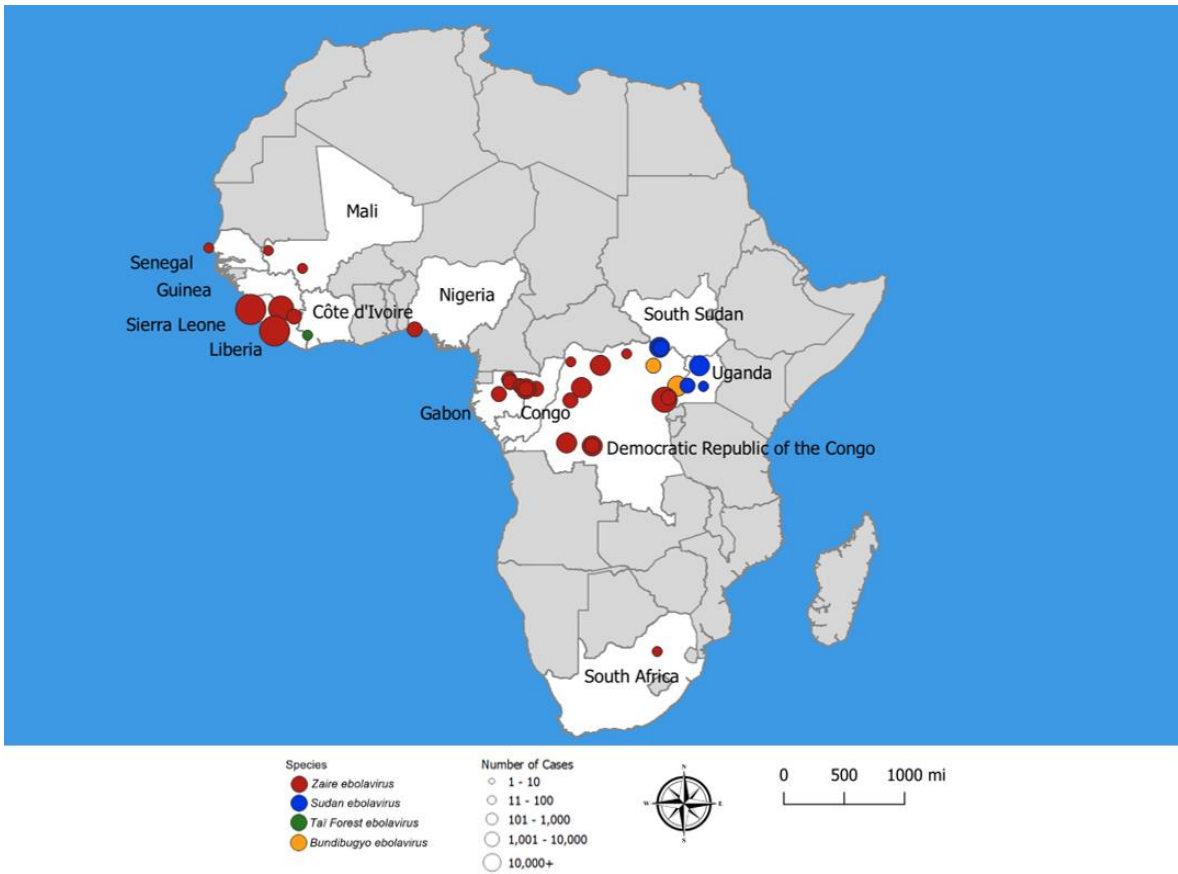


Figure 8. Geographic distribution of Ebola virus outbreaks since 1976 (CDC, 2021a).

Table 2. Ebola Virus Outbreaks by Species and Size, Since 1976.

	Country*	Cases	Deaths	Species	Year
1.	Uganda	-	-	<i>Sudan ebolavirus</i>	2022
2.	Dem. Rep of the Congo	1	1	<i>Zaire ebolavirus</i>	2022
3.	Dem. Rep of the Congo	10	10	<i>Zaire ebolavirus</i>	2022
4.	Dem. Rep of the Congo			<i>Zaire ebolavirus</i>	2021
5.	Guinea	23	12	<i>Zaire ebolavirus</i>	2021
6.	Dem. Rep of the Congo	12	6	<i>Zaire ebolavirus</i>	2021
7.	Dem. Rep. of the Congo	130	55	<i>Zaire ebolavirus</i>	2020
8.	Dem. Rep. of the Congo, Uganda	3470	2287	<i>Zaire ebolavirus</i>	2018-2020
9.	Dem. Rep. of the Congo	54	33	<i>Zaire ebolavirus</i>	2018
10.	Dem. Rep. of the Congo	8	4	<i>Zaire ebolavirus</i>	2017
11.	Italy	1	0	<i>Zaire ebolavirus</i>	2015
12.	Spain	1	0	<i>Zaire ebolavirus</i>	2014
13.	UK	1	0	<i>Zaire ebolavirus</i>	2014
14.	USA	4	1	<i>Zaire ebolavirus</i>	2014
15.	Senegal	1	0	<i>Zaire ebolavirus</i>	2014
16.	Mali	8	6	<i>Zaire ebolavirus</i>	2014
17.	Nigeria	20	8	<i>Zaire ebolavirus</i>	2014
18.	Dem. Rep. of the Congo	66	49	<i>Zaire ebolavirus</i>	2014
19.	Sierra Leone	14124 [#]	3956 [#]	<i>Zaire ebolavirus</i>	2014
20.	Liberia	10675 [#]	4809	<i>Zaire ebolavirus</i>	2014
21.	Guinea	3811 [#]	2543 [#]	<i>Zaire ebolavirus</i>	2014
22.	Uganda	6	3	<i>Sudan ebolavirus</i>	2012
23.	Dem. Rep. of the Congo	36	13	<i>Bundibugyo ebolavirus</i>	2012
24.	Uganda	11	4	<i>Sudan ebolavirus</i>	2012
25.	Uganda	1	1	<i>Sudan ebolavirus</i>	2011
26.	Dem. Rep. of the Congo	32	15	<i>Zaire ebolavirus</i>	2008
27.	Uganda	149	37	<i>Bundibugyo ebolavirus</i>	2007
28.	Dem. Rep. of the Congo	264	187	<i>Zaire ebolavirus</i>	2007
29.	Congo	12	10	<i>Zaire ebolavirus</i>	2005
30.	Sudan (present day South Sudan)	17	7	<i>Sudan ebolavirus</i>	2004
31.	Republic of Congo	35	29	<i>Zaire ebolavirus</i>	2003
32.	Republic of Congo	143	128	<i>Zaire ebolavirus</i>	2002
33.	Republic of Congo	57	43	<i>Zaire ebolavirus</i>	2001
34.	Gabon	65	53	<i>Zaire ebolavirus</i>	2001-2002
35.	Uganda	425	224	<i>Sudan ebolavirus</i>	2000
36.	South Africa	2	1	<i>Zaire ebolavirus</i>	1996
37.	Gabon	60	45	<i>Zaire ebolavirus</i>	1996

38.	Gabon	37	21	<i>Zaire ebolavirus</i>	1996
39.	Zaire (present day DRC)	315	250	<i>Zaire ebolavirus</i>	1995
40.	Côte d'Ivoire (Ivory Coast)	1	0	<i>Tai Forest ebolavirus</i>	1994
41.	Gabon	52	31	<i>Zaire ebolavirus</i>	1994
42.	Sudan (present day South Sudan)	34	22	<i>Sudan ebolavirus</i>	1979
43.	Zaire (present day DRC)	1	1	<i>Zaire ebolavirus</i>	1977
44.	Sudan (present day South Sudan)	284	151	<i>Sudan ebolavirus</i>	1976
45.	Zaire (present day DRC)	318	280	<i>Zaire ebolavirus</i>	1976

*Source: (CDC, 2021a; WHO, 2021).

#Include suspect, probable and confirmed EVD cases.

2.2.1.1. Epidemiology of *Zaire Ebolavirus*

Zaire ebolavirus (EBOV) is the deadliest among the four strains of Ebola virus involved in different outbreaks in humans. The first outbreak involving *Zaire ebolavirus* was reported in 1976 in DRC with a total number of 318 cases and 280 deaths which leads to fatality rate of 88% (WHO, 2021). Prior to the largest recorded *Zaire ebolavirus* outbreak in West Africa in 2014-2016 with 28,610 cases, and the 2018-20 outbreak in the Democratic Republic of the Congo with 3,470 cases, the virus was implicated in several outbreaks in Republic of the Congo (2001, 2002, 2003), DRC (1976, 1977, 1995, 2007, 2008, 2014, 2014-2016, 2017, 2018, 2018-2020, 2020, 2021, 2022), Uganda (2018-2020), Gabon (1996), and South Africa (1996) (CDC, 2021a). During these events, there were cases that have been transported to other countries called “imported cases”, e.g., the South Africa government reported one case of EVD from a medical professional who traveled from Gabon to South Africa, after treating EVD patients in Gabon. He recovered from the disease while the nurse who was treating him got infection and died (CDC, 2022b). During the West Africa outbreaks, imported cases were recorded in Europe and also in the USA. Simultaneously with the 2018-2020 EVD outbreak in eastern DRC, imported cases of EVD were recorded in Uganda. Four cases have been reported without local transmission of the virus, suggesting that population displacement, international travel, globalization are the factors can lead to the expansion of the virus (WHO, 2022a).

2.2.1.2. Epidemiology of *Sudan ebolavirus*

Sudan ebolavirus (SUDV) has been implicated in one of the first two outbreaks caused by Ebola virus. A total of 284 cases and 151 deaths occurred in South Sudan, with a mortality rate of 53%. The outbreak is believed to have started in a cotton factory where 37% of workers were infected, and the virus later spread mainly through close personal contact within hospital staff (Sivanandy *et al.*, 2022; WHO/International Study Team, 1978a). Apparently less lethal than EBOV, SUDV has caused several outbreaks after its initial registration. An outbreak reported in the towns of Nzara and Yambio, Sudan in 1979 caused 34 cases with a 65% case fatality rate (Baron *et al.*, 1983; CDC, 2022b). Furthermore, four additional outbreaks caused by SUDV were registered in Uganda (2000, 2011, 2012), and Sudan (2004). The 2000 Ebola outbreak in Uganda was the largest one caused by SUDV. The outbreak started in the region of Gulu and spread later to the region of Masindi and Mbarara with 425 cases reported, and 224 (53%) deaths. (CDC, 2022b). To control the epidemic, local and international response committees have harmonized the implementation of a jointly agreed program. Therefore, community mobilization and political bodies such as Members of Parliament have been used to educate the public to avoid spreading rumors and misinformation (Okware *et al.*, 2002). The management of information and rumor is one of the principal actions during the implementation of any outbreak response program.

2.2.1.3. Epidemiology of *Bundibugyo ebolavirus*

Bundibugyo ebolavirus (BDBV) was first discovered in 2007 during a hemorrhagic fever outbreak (EBOV-like) in Uganda's Bundibugyo district. The outbreak started in August 2007, and 131 (44 suspect, 31 probable, and 56 confirmed) cases were identified with 42 (32%) of deaths (MacNeil *et al.*, 2011; MacNeil *et al.*, 2010). During the outbreak, eight samples from suspected EVD patients were positive by antigen capture assay that shows high cross-reactivity between different strains of the Ebola virus genus. However, these samples were negative when using highly sensitive retro-transcription polymerase chain reaction (RT-PCR), which is specific for EBOV, SUDV, and Marburgvirus. Meanwhile, further analysis using a broadly reactive filo virus L gene-specific RT-PCR showed a positive sample, and sequence analysis of the polymerase chain reaction (PCR) product (400 bp of viral L gene) showed that the virus was different from the four previously

identified strains of Ebola virus, suggesting that it was a new Ebolavirus variant and was named Bundibugyo in reference to the district where the virus caused the outbreak (Kozak *et al.*, 2016; Towner *et al.*, 2008). The virus was also involved in the 2012 hemorrhagic fever outbreak which took place in the Oriental province located in the northeast of the DRC, and caused 38 laboratory confirmed cases with 13(34%) of reported number of deaths (CDC, 2022b). Today only 2 outbreaks involving BDBV have been observed.

2.2.2. Symptoms and clinical evolution

It is largely known that infection by Ebola virus is usually followed by symptoms after an incubation period which is globally accepted to be in a range of 2 to 21 days with an average of 6.22 ± 1.5 days (Jacob *et al.*, 2020). After the incubation period, the natural evolution of EVD can be divided in two phases: early phase and the peak phase (Jacob *et al.*, 2020). The early phase (1 to 3 days following disease onset) is generally a non-specific febrile illness resembling many infectious diseases in sub-Saharan Africa that usually include headache, fever, chills, arthralgia, myalgia, malaise, anorexia, and rash (Leligdowicz *et al.*, 2016). During the peak phase of EVD, which takes place 4 to 6 days after the disease onset, patients usually develop gastrointestinal symptoms (nausea, vomiting, abdominal pain, and diarrhea) as the viral load increases. This phase also called humid phase, can lead to the clinical complications such as hypovolemia and shock with multisystem organ dysfunction (Barry *et al.*, 2015; Jacob *et al.*, 2020). Diarrhea was found to be significantly associated with a fatal outcome along with weakness, and dizziness in Sierra Leone (Schieffelin *et al.*, 2014). Moreover, hemorrhage which is one of the main clinical manifestations from the upper or lower gastrointestinal tract or both occurred before death in less than 5% (Chertow *et al.*, 2014). In a study from Guinea, hemorrhage was present in 38.5% of deceased patients and 16% of surviving patients, suggesting that hemorrhage does not always occur in EVD patients even in those who die (Barry *et al.*, 2015).

2.2.3. Studies on asymptomatic or pauci-symptomatic Ebolavirus disease

Ebolavirus disease is known to be a severe clinically symptomatic disease with high mortality rate. However, the studies conducted during the 2014-2016 EBOV outbreak in West Africa have reported serological evidences that asymptomatic and pauci-symptomatic

clinical disease also occur. Glynn et al., reported that 12% of EVD contacts that had few symptoms at the time of their exposure were seropositive to Ebola GP antigen, and 2.6% of seropositive among asymptomatic contacts (Glynn *et al.*, 2017). Richardson et al., have reported that 14 out of 187 participants from a EVD hotspot village in Sierra Leone were positive to EBOV antigens, suggesting that they have been exposed to EBOV during the outbreak but they did not be diagnosed (Richardson *et al.*, 2016). Diallo et al., reported 18% out of 216 paucisymptomatic contacts and 3.2% out of 1174 asymptomatic participants seropositive to EBOV antigens (Diallo *et al.*, 2019). Likewise, asymptomatic EBOV infection was reported in 11 of 24 asymptomatic individuals during the previous recorded EVD epidemic in 1996, in Gabon. Serological markers (anti-EBOV IgM and IgG) were detected among them. The authors have documented also the presence of the RNA of EBOV in peripheral blood mononuclear cells (Leroy *et al.*, 2000).

The above studies of asymptomatic or pauci-symptomatic Ebola virus infection have some limitations. In fact, most of these studies were conducted after the end of the different outbreaks, so they could likely report information with recall bias. Another limitation is that a wide diversity of antibody detection assays that have been applied in most of these studies, used a single antigen, (GP) or viral lysates, the glycoprotein known to be cross-reactive with other viruses. Finally, none of these studies evaluated the rate of symptomatic or pauci-infection in suspects patients diagnosed as negative after two consecutive Ebola RT-PCR tests during the outbreak.

2.2.4. Transmission pathways

EBOV is mostly transmitted between humans through direct contact and indirect contact with objects contaminated by infected bodily fluids. Of these, blood, cerebrospinal fluid, and breast milk are the most contagious while other fluids such as semen, saliva, urine, aqueous humor, amniotic fluid, and skin swab have been recognized carrying EBOV (Dowell *et al.*, 1999; Galas, 2014; Roels *et al.*, 1999). Indeed, the re-emergence of EBOV in surviving patients, reported in the Democratic Republic of Congo during the 10th EVD outbreak and in Guinea during the EVD outbreak in 2021, highlighted the role of human fluids such as semen, aqueous humor and breast milk. where the virus can remain in an

inactivated form for a long time, and consequently the reactivation of the quiescent virus can lead to the genesis of a new outbreak (Keita *et al.*, 2021; Mbala-Kingebeni *et al.*, 2021).

2.2.5. Ebola virus immune response

2.2.5.1. Innate immunity

Immune cells such as macrophages, monocytes and dendritic cells are the main targets of EBOV after the virus crosses the skin barrier. Infected cells that are part of the innate immune system produce inflammatory response that is accompanied by the release of several cytokines. Among them, type 1 IFN (IFN α/β) was found to be critical for the outcome in infected mice (Bray, 2001; Leroy *et al.*, 2000). However, some EBOV antigens interfere with the innate immune response by blocking or damping the release of cytokines. For example, VP35 blocks the phosphorylation of the IFN-regulatory factor 3 (IRF3), which is important for the transcription of the genes that encode for the production of IFN. Likewise, VP24 block the IFN signaling, and consequently, innate immune response fails to contain the infection spread to uninfected cells (Hoenen *et al.*, 2006). Moreover, DCs also fail to produce pro-inflammatory cytokines and lose their ability to support T-cell proliferation and undergo abnormal maturation leading to impaired cellular immune response (Bosio *et al.*, 2003). Ultimately, the number of Natural Killer (NK) cells dramatically drops during EBOV infection in non-human primates while these cells seem to be not infected by EBOV (Tosi, 2005). These cell seem to undergo apoptosis as they express high levels of caspase activity (Geisbert *et al.*, 2000; Reed *et al.*, 2004).

2.2.5.2. Adaptive immunity

We showed above that EBOV impairs the function of DCs, which are playing an important role between innate and adaptive immune responses. Indeed, DCs use pattern recognition receptor (PRR) to recognize viral antigen and release as immune mediators leading to the maturation of DCs that will finally downregulate proteins involved in phagocytosis and upregulate proteins involved in antigen presentation and cell mobility after pathogen processing (Mahanty *et al.*, 2004). Nevertheless, the infection of DCs by EBOV result in impairing of innate and adaptive immune responses, e.g., macrophage inflammatory protein 1 α (MIP-1 α) and monocyte chemotactic (MCP-1) are released as mediator but not IFN α ,

Interleukin 8 (IL8) and TNF α (Kelsall *et al.*, 2002). Consequently, MCP and IL8 are playing to recruit macrophages to the site of viral replication for the containment of the infection while the inhibition of the expression of IFN α/β through EBOV VP35 and VP24 will contribute to survival and dissemination of the virus (Calder *et al.*, 2003).

During natural EBOV infection, antibodies are released early in the course of the infection, between 2 and 9 days for IgM and between 6 and 19 days for IgG, and patients who survive from the illness are supposed to develop antibodies with ability to persist for several years in blood (Figure 9) (Rimoin *et al.*, 2018; Rowe *et al.*, 1999). Although the role of the human adaptive immune response in EBOV infection is still difficult to assess due to the lack of relevant data from animal model, the reported differences observed in the adaptive response between fatal and non-fatal cases show that the adaptive immune response against EBOV plays an important role in controlling the infection (Baize *et al.*, 1999; Hoenen *et al.*, 2006; Sanchez *et al.*, 2004). Several studies assessed the presence of the specific EBOV antibodies among high risk populations, close EVD contacts, and EBOV survivors in order to characterize the EBOV immune response (Diallo *et al.*, 2021; Diallo *et al.*, 2019; Keita *et al.*, 2018; Keita *et al.*, 2019; Mbala *et al.*, 2017; Mulangu *et al.*, 2016). Due to possible cross reaction in serological tests used, antibody results should be interpreted with caution, in order to not overestimate the seroprevalence like that have been observed in many of the assessments reported above. Thus, it is important to use stringent positivity criterion like that was done in Guinea and reported by Diallo *et al.* in order to avoid unspecific reactivity that can introduce bias. Indeed, three viral antigens of EBOV have been used in multiplex assay and a simultaneous positivity to at least two antigens was used as positivity criteria for the determination of seroprevalence in asymptomatic and pauci-symptomatic patients (Diallo *et al.*, 2019).

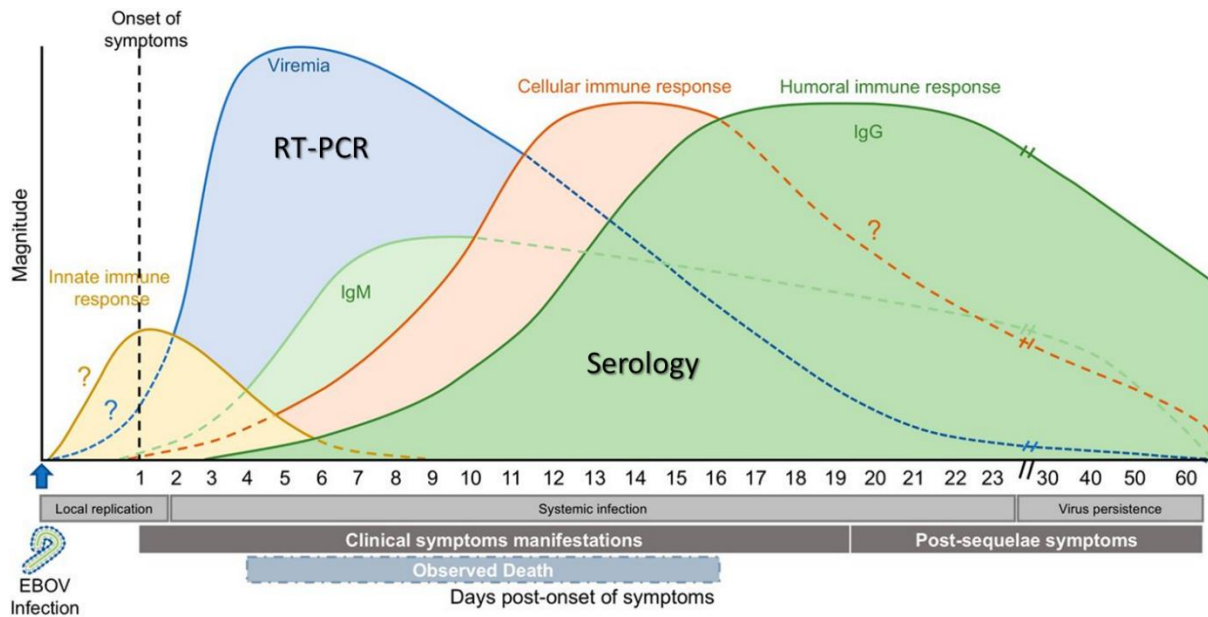


Figure 9. Antigen and antibodies kinetic during EBOV infection. Adapted from

EBOV replicates locally and triggers innate immune responses (yellow). The increase in viraemia in the systemic circulation (in blue) corresponds to the self-reported appearance of symptoms leading to hospitalization of an infected person several days after the onset of symptoms. The virus can be detected or isolated. At the symptom onset, IgM (light green), IgG (dark green) and cellular (orange) responses are detected. During the healing phase, the virus can persist in immunoprivileged sites in the absence of viraemia (Ploquin *et al.*, 2018).

2.2.6. Studies on the kinetic of anti-Ebola antibodies in survivors

Antibodies measurement play a crucial role in understanding and managing a highly immunogenic viral disease with a very high mortality rate such as EVD. Indeed, early production of antibodies at high levels in infected patients with Ebola virus appears to be associated with good patient outcomes and increased probabilities of survival (Baize *et al.*, 1999; Ksiazek, Rollin, *et al.*, 1999; McElroy *et al.*, 2015). Thus, several researchers have looked into this question in order to be able to characterize the anti-Ebola immune response in survivors. Based on examples of other viruses such as yellow fever, it was hypothesized that antibody production would be ad-Vitam after survival from EVD, e.g., a study conducted among EVD survivors from the first Yambuku outbreak in 1976 in the DRC reported that 7 out of 14 survivors showed antibody reactivity to EBOV-GP, while 3

patients who were classified as confirmed cases during the acute disease, have presented neutralizing antibody activities 40 years after infection (Rimoin *et al.*, 2018).

At the beginning of our thesis project, we have revised several studies that evaluated the long-term kinetics of anti-Ebola antibodies in survivors. Among them, a study conducted in 4 EVD survivors treated at Emory University Hospital, Atlanta, Davis *et al.*, reported a convergent evolution of neutralizing antibody production during the first three years of follow-up, suggesting that EVD survivors can be protected against reinfection during this documented period (Davis *et al.*, 2019). In another study conducted on a cohort of EVD survivors in Guinea, Thom *et al.* reported a mean neutralizing antibody titer of 1/174 in survivors that provided samples between 3 and 14 months after infection. They have also observed that antibody titers continuously increased over time (2 years of follow-up) and were significantly associated with the presence of the cellular immune response represented by the T-cell assay, suggesting the possibility of a long-term protective immune response in EVD survivors (Thom *et al.*, 2021). Furthermore, Adaken *et al.*, who followed a cohort of 117 EVD survivors in Sierra Leone reported a rapid decline in GP antibody levels, unlike previous studies. They developed a pharmacodynamic model of antibody reactivity that identified a decay half-life of 77-100 days and a doubling time of 46-86 days in a high percentage of survivors. The highest antibody reactivity was observed approximately 200 days after infection. Thus, the observed up and down antibody kinetics suggests the presence of a source of antigenic re-stimulation in survivors who may continue to carry the virus in the anatomic immune-privileged regions (Adaken *et al.*, 2021). More recently, Diallo *et al.*, have followed-up for 60 months a large cohort of 802 EVD survivors in Guinea, where they used a serological multiplex assessment longitudinal seropositivity to EBOV antigens. They reported an overall significant decrease on anti-Ebola antibodies overtime of 25%, particularly for those produced against nucleoproteins, and the persistence of EBOV RNA in semen was significantly associated with high concentrations of antigens against NP that could explain the observed individual antibody wax-and-wane pattern (Diallo *et al.*, 2021).

The above reported data on the kinetics of antibodies against Ebola virus in EVD survivors are variable and underline the importance of being able to continue studies for a better understanding of EVD. In fact, the measurement and the characterization of antibody

kinetics in EVD survivors plays an important role as landmark for the evaluation of the efficacy of candidate vaccines, including that have been used in clinical trials during the West Africa EVD outbreak and at large scale during the 10th outbreak of EVD in the DRC. Beside use of vaccines, specific treatments including three monoclonal antibodies and an antiviral agent have been used during the 10th EVD outbreak in DRC. Therefore, it is useful to continue studies evaluating the anti-Ebola humoral immune response in survivors in order to understand the impact of these new treatments on antibody kinetics compared to previous studies performed on survivors who had not received specific treatment.

2.2.7. Laboratory diagnosis

EBOV is classified as a biosafety level 4 agent due to its high mortality rate and lack of approved vaccines and antivirals with full protection. However, most EVD outbreaks have occurred in remote areas where healthcare facilities and qualified personnel are lacking, making it difficult to implement diagnostic platforms (CDC, 2022b). Since the virus was discovered in 1976 using traditional viral culture techniques and electron microscopy, the laboratory diagnosis of EBOV has been improved overtime.

2.2.7.1. Cell culture and electron microscopy

The traditional isolation of EBOV in cell culture using Vero E6 African Green monkey kidney cells is recognized as the gold standard method. Once the virus infects Vero cells that are receptive for the virus, the cytopathogenic effect can be visualized directly using electron microscopy or indirectly using immunofluorescence microscopy (Bowen *et al.*, 1977; Johnson *et al.*, 1977). However, the cell culture method can only be performed in Biosafety Level 4 (BSL-4) containment and is only recommended for research and public health laboratories, and is thus almost impossible to implement in the field. EBOV detection using cell culture can be done in one to five days (Broadhurst *et al.*, 2016).

2.2.7.2. Molecular diagnosis

Conventional RT-PCR. This method was developed by the CDC and first evaluated on the samples collected during the 1995 EVD outbreak in Kikwit/DRC. After the extraction of the viral RNA, the PCR was used to amplify the GP, NP, and L genes, and the target size-

based amplicon was detected using gel electrophoresis (Sanchez *et al.*, 1999). Compared to EBOV antibody and antigen detection, conventional RT-PCR shows higher clinical performance and, interestingly, is able to detect infection at an early stage of its development as well as in other body fluid such as saliva and semen (Sanchez *et al.*, 1999).

Real-time RT-PCR. First, the method was developed by USAMRIID in 2001, and then evaluated by CDC using left-over samples collected during the 2000 EVD outbreak in Uganda previously characterized by ELISA and conventional RT-PCR (Drosten *et al.*, 2002; Gibb *et al.*, 2001; Towner *et al.*, 2004). The real-time RT-PCR provides information about the viral copy numbers using cycle threshold (C_T) values where the conventional RT-PCR does not. Indeed, the estimation of viral copy number using C_T values was reported in correlation with the quantification of viral load by plaque assay (Towner *et al.*, 2004). Another advantage of the real-time RT-PCR resides on the fact that this method uses sequence-specific probes that provide higher clinical performance and rapid results than in conventional detection method (Sanchez *et al.*, 1999).

Real-time RT-PCR using Xpert® Ebola Assay. Because of the lack of adapted facilities for the above-mentioned techniques in African remote areas, a molecular point-of-care (POC) test, the Xpert® test (Cepheid, Sunnyvale, CA, USA), was developed for the detection of EBOV in blood and subsequently in a large diversity of other bodily fluids. The assay has advantages of higher limits of detection rate (approximately 275 copies/mL), possibility of to be implemented in areas with limited laboratory resources, infrastructure, and less trained personnel. The test also allows rapid results after sampling (100 minutes), and increases operator's safety as all steps of the PCR reaction are contained in a single reaction (Cepheid, 2022). The test was first evaluated during the 2014-2016 West African EBOV outbreak in comparison with the traditional molecular RT-PCR and has shown a sensitivity and specificity close to 100% in different bodily fluids (Loftis *et al.*, 2017; Pettitt *et al.*, 2017; Pinsky *et al.*, 2015; Semper *et al.*, 2016). Moreover, the Xpert® tests were deployed in the 2018 EVD outbreak in Equateur in DRC, but also in ten mobile laboratories deployed on the field during the 10th EVD outbreak in Ituri and North Kivu in DRC (Mbala-Kingebeni *et al.*, 2019). This test therefore effectively contributed to the containment of an EVD outbreak in the DRC.

Molecular evolution by sequencing. Ebola virus strains are sequenced to identify the source of outbreak, virus transmission routes, and dynamics of the epidemic. Indeed, sequencing highlights the genetic structure of the virus and makes it possible to compare the genome with other strains already known, in order to better understand its evolution ([Broadhurst *et al.*, 2016](#)). For example, sequencing made it possible to understand that the viruses responsible for the epidemic in West Africa were caused by the EBOV strain, but that the latter was not directly linked strains isolated in Central Africa, suggesting different spill-over events from a naturally zoonotic reservoir of EBOV ([Gire *et al.*, 2014](#)). In the DRC, sequencing was widely used during the 10th EVD outbreak in the east of the country ([Mbala-Kingebeni *et al.*, 2019](#)). Indeed, the sequencing data were important to complete the chains of contamination around the confirmed cases, which were difficult to investigate due to the political instability and civil war in the region and the community resistance to the response activities. During this epidemic, the Next-Generation Sequencing (NGS) platforms were used to carry out the sequencing, and considering the fact that they are easy to implement even in remote places, the sequencing laboratory had been deployed in the field and analyzes were carried out there.

2.2.7.3. Serological assays

Detection of specific antibodies against EBOV. An immune fluorescence method was first developed for the detection of antibody against Lassa virus, and then adapted for EBOV antibody detection during EBOV outbreak in the DRC in 1976. The test aimed to identify current and past EBOV infection in suspected patients ([WHO/International Study Team, 1978b](#); [Wulff *et al.*, 1975](#)). Viral antigens from cultures are irradiated, fixed on a slide and incubated with the serum sample, and finally bound antibodies are detected with a fluorescently labeled secondary antibody ([Wulff *et al.*, 1975](#)). However, the IFA method requires BSL-4 facilities and has suboptimal sensitivity and specificity, limiting it to only research and public health laboratories ([Broadhurst *et al.*, 2016](#)). Thus, the enzyme-linked immunosorbent assay (ELISA) was then developed to detect specific IgM and IgG antibodies against EBOV. Although ELISA could be performed in BSL-2 facilities, viral inactivation by gamma irradiation, still required for the inactivation of viral cultures and samples, was only available in some laboratories, limiting its implementation ([Ksiazek, West, *et al.*, 1999](#)). Additionally, ELISA using recombinant EBOV proteins has been

performed for research purposes as well as in commercial kits that can also be implemented in resource-limited conditions and countries (Saijo *et al.*, 2006). Finally, antibody detection is of little use in the diagnosis of EBOV. In fact, IgM cross-reacts less with other filoviruses, whereas IgG appears to do so, and antibodies are detected between days 2-10 after appearance of symptoms for IgM and days 6-19 for IgG (Rowe *et al.*, 1999), but are important for the determination of residual immunity and vaccine evaluation (Jacob *et al.*, 2020). More and more multiplex tests using magnetic beads and the technique of flow cytometry are being developed to optimize the evaluation of seropositivity in the context of possible cross-reactions.

Detection of EBOV antigens. The sandwich ELISA for the detection of EBOV antigens was developed in 1989 at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). The kit uses a pool of 8 mouse monoclonal antibodies to recognize SUDV and EBOV epitopes for antigen capture and rabbit polyclonal antibodies for antigen detection (Ksiazek *et al.*, 1992). The antigen-based ELISA was first evaluated during the 1995 EVD outbreak in Kikwit/DRC. The test showed acceptable clinical performance (detection of antigen by day 3 of disease onset) and was performed faster than those used previously (~ 5h). It was also used in the field during the 2000 EVD outbreak in Gulu/Uganda and was suggested to be used as the standard diagnostic test (Ksiazek, Rollin, *et al.*, 1999; Towner *et al.*, 2004). Recently, lateral flow tests for the detection of EBOV antigen in the field have been evaluated showing variable performances. QuickNavi™-Ebola showed a sensitivity of 85% and a specificity of 99.8% in comparison with GeneXpert confirmed cases. However, OraQuick Ebola test showed 100% of sensitivity and 2% of false positives in post-mortem samples analyzed compare to GeneXpert results (Makiala *et al.*, 2019; Daniel Mukadi-Bamuleka *et al.*, 2022; D. Mukadi-Bamuleka *et al.*, 2022). The advantage of these tests, is that they can be done on site without equipment and provide rapidly results.

2.2.8. Treatment

2.2.8.1. Prevention

As explained above, EBOV transmission starts generally by spillover event and then spreads from the index case to other receptive individuals. Nevertheless, social dynamics and environment of where the outbreak occurs can greatly influence the perception of the event by local health care personnel and the general population. Therefore, it is important to understand the cultures and behavior of the local community in order to find optional strategies to contain the spread of the virus, while establishing trust and good communication with the affected communities ([Muzembo et al., 2020](#)).

General measures can be implemented during an epidemic in order to stop the virus spread ([CDC, 2022b](#)). Among them we can cite:

- Avoid contact with blood and body fluids (such as urine, feces, saliva, sweat, vomit, breast milk, amniotic fluid, semen, and vaginal fluids) of people who are sick.
- Avoid contact with semen from a man who has recovered from EVD, until testing shows that the virus is gone from his semen.
- Avoid contact with items that may have come in contact with an infected person's blood or body fluids (such as clothes, bedding, needles, and medical equipment).
- Avoid funeral or burial practices that involve touching the body of someone who died from EVD or is suspect for EVD.
- Avoid contact with bats, forest antelopes, and nonhuman primates (such as monkeys and chimpanzees, gorillas) blood, fluids, or raw meat prepared from these or unknown animals (bushmeat).
- Isolation of individuals with suspected, probable or confirmed EVD cases for doing contact tracing, follow-up over 21 days, and treatment of confirmed cases.

Vaccination. There has been a need for developing vaccine that can prevent against EBOV while the few numbers of EVD cases and the remotely locations of many of the outbreaks that occurred before the 2014 West African outbreak did not favor vaccine development. EBOV vaccine development started shortly after the first EVD outbreak in 1976. First, vaccine-based formalin-fixed or heat-inactivated viruses were used in a group of seven

guinea pigs and in non-human primates for the evaluation of the vaccine efficacy and the level of protection against disease. Vaccines could not totally protect guinea pigs against inoculated Ebola virus (Lupton *et al.*, 1980), and in another study that used baboons for experiment, up to 90% of baboons survived against the infection (Mikhailov *et al.*, 1994). Over the past decade, combinations of DNA immunization and boosting using adenoviral vectors encoding viral proteins that induce cellular and humoral immunity in cynomolgus macaques have been developed and evaluated in non-human primates. A group of four vaccinated macaques survived after challenging with a lethal dose of the wild-type Mayinga strain from the EBOV (Sullivan *et al.*, 2000), suggesting that vaccines can be used as preventive treatment against Ebola virus infection.

The unprecedented major EVD outbreak of 2014-2016 in West Africa highlighted the need to accelerate the development and evaluation of candidate EVD vaccines. Many candidate vaccines are based on EBOV GP because GP is known to elicit a strong immune response and neutralize antibodies (Bhatia *et al.*, 2021). The advanced vaccine candidate in clinical trials and currently approved by the U.S. Food and Drug Administration (FDA), rVSVΔG-ZEBOV-GP (called Ervebo®), a live attenuated recombinant vesiculovirus, was used in the DRC during the 2018 EVD outbreak in a ring vaccination strategy, consisting of vaccinating EVD patient contacts, contacts of contacts, and frontline health care professionals. Evaluation of the vaccine candidate showed that at 21 days of follow-up, 87.2% had an antibody response and 95.6% had antibody persistence at 6 months of follow-up (Hoff *et al.*, 2022). The vaccine was approved by the U.S. FDA on December 19, 2019 as the first FDA-approved vaccine for Ebola. It is given as a single dose vaccine and has been found to be safe and protective against Zaire ebolavirus, which has caused the largest and most deadly Ebola outbreaks to date (CDC, 2022b). Furthermore, the Johnson and Johnson experimental Ad26.ZEBOV/MVA-BN-Filo vaccine has been used as a second option in curtain strategy. People who lived in non-EBOV affected regions around EBOV-affected regions received two doses of the J&J vaccine after giving their consent (WHO, 2019a). Indeed, it is important to continue evaluating vaccines that have already been approved and to develop new vaccines to improve efficacy and safety against Ebola. Therefore, other vaccine candidates are still being evaluated in randomized trials, such as the cAd3 EBO vaccine licensed by the Sabin Vaccine Institute. This vaccine uses

chimpanzee adenovirus (cAd3) as a vector to deliver the genetic material of the Ebola virus. The vaccine has been shown to be effective in several clinical trials, including a field study in Liberia (PREVAIL 1) (NIH, 2020).

2.2.8.2. Convalescent plasma and plasma therapy

Due to high mortality rate from Ebola observed in 1976, but also the lack of evidence for therapeutic interventions before and after exposure at that time, there was a need to develop preventive vaccines and antiviral therapies to protect people against EBOV infection. As described above, and taking into account evidence from animal models such as rodents, the production of high-quality neutralizing antibodies can protect patients at the time of disease onset and limit the severity of the disease without possibly curing it completely (Feldmann *et al.*, 2003). Based on this hypothesis, recovery plasma from EBOV survivors that is supposed to contain neutralizing antibodies has been proposed for the treatment of patients with acute EVD. Due to the limited number of EVD survivors at the time, hyperimmune horse serum was developed, which has been shown to protect baboons from experimental infection with EBOV (Mikhailov *et al.*, 1994), as well as guinea pigs, while it did not protect *Cynomolgus* monkeys during EBOV infection (Jahrling *et al.*, 1996). Plasma transfusion was used in humans during the 1995 Ebola virus disease outbreak in Kikwit, DRC, where 8 confirmed patients were transfused with convalescent plasma from 5 recovering patients, and only one patient (12.5%) died, suggesting convalescent plasma may protect against EBOV (Mupapa *et al.*, 1999). In a nonrandomized, comparative study conducted during the 2014-2015 EVD outbreak in Guinea, 84 patients received up to 500 ml of convalescent plasma with unknown levels on neutralizing antibodies. the risk of death was 31% in the treatment group and 38% in the control group (418 patients who did not receive plasma transfusion). This trial has shown that convalescent plasma was not associated with a significant improvement in survival among confirmed EVD patients (van Griensven *et al.*, 2016). Although plasmapheresis efficacy was difficult to evaluate because of several unknowns such as evolution of neutralizing antibodies in plasma donors, the use of plasma could have conveyed other healing factors that may be important in patients who have presented hemorrhage during acute phase (Garraud, 2017). Thus, the plasmapheresis hypothesis that consist in the assumption of protection induced by neutralizing antibodies

has been used as precursor of newly developed monoclonal antibodies for the treatments of several infections and non-infectious diseases.

2.2.8.3. Antiviral treatment

The West African Ebola outbreak was the largest outbreak caused by EBOV ever recorded that occurred also in an urban setting with a rapid spread in the population. However, there were no available and proven safe or effective therapies or vaccines that could be used for treatment of EVD patients while several experimental antiviral treatments were under development and evaluation in trials. Therefore, there has been debate on the ethical considerations for using experimental therapies in rigorous clinical trials during the epidemic. Indeed, the panel members concluded that disease outbreaks are not a good setting to conduct rigorous clinical trials. Because when people are dying, all possible therapies should be given a chance, rather than studied in rigorous ways. Consequently, antiviral molecules were used in several small trials during the EVD outbreak in West Africa, with often poor and inconclusive results ([Lane et al., 2021](#)). Taking into account these results, the US National Academies of Sciences, Engineering, and Medicine recommended that randomized clinical trials are the most reliable methods to determine the relative benefits of treatment and subsequent risks of candidate medicines ([National Academies of Sciences et al., 2017](#)). Thereby, during the 2018–2020 EVD outbreak in the DRC, four available candidate-specific molecules that have proven efficacy in experimental animal studies to protect against EBOV were evaluated in a rigorous randomized clinical trial. There were three monoclonal antibodies targeting the viral glycoproteins, Ansuvimab, REGN-EB3 and ZMapp, and an antiviral, Remdesivir, which appears to inhibit viral replication of EBOV RNA ([Diakou et al., 2021](#)). Among these, Ansuvimabb and REGN-EB3 were significantly associated with higher survival rate of EVD patients which was 90% ([Mulangu et al., 2019](#)). Following the evidence of anti-Ebola drug's efficacy, two treatments were approved by the US FDA for the treatment of EVD in adults and children caused by the EBOV infection. Inmazeb™ (Inmazeb), the first drug approved in October 2020, is a combination of three monoclonal antibodies. The second drug, Ebanga™ (Ansuvimab), was approved in December 2020 is a single monoclonal antibody. Indeed, monoclonal antibodies act like natural antibodies, preventing the virus from replicating after infecting a person. These mAbs bind to viral glycoproteins that prevent the virus from

attaching to and entering the target cell ([CDC, 2022b](#)). Although these molecules have been approved, further investigations are necessary for evaluating late adverse event that could be correlated to the treatment used, but also to evaluate immunological outcomes of patients treated with these molecules as immune responses may have an impact on the probability of disease relapse in a subset of patients who continue to carry the virus in immune privileged sites.

Chapter 3. Severe Acute Respiratory Syndrome Coronavirus 2

This chapter will give an insight on the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for the current pandemic of coronavirus disease (COVID-19).

3.1. History of SARS-CoV-2

In early December 2019, cases of pneumonia of unknown cause occurred in Wuhan, Hubei Province, China (Del Rio *et al.*, 2020). In the first epidemiological investigation of a cluster of pneumonia cases of unknown aetiology, including seven severe cases reported in late December 2019, twenty-seven cases were associated with the South China seafood wholesale Market of Wuhan. The market was disinfected and closed to the public on January 1, 2020. Contact tracing was performed and allowed to identify additional 160 close contacts of the reported cases. These contacts were under medical surveillance until they developed or not the disease (Wu *et al.*, 2020). Researchers at the Wuhan Institute of Virology performed metagenomic RNA sequencing using a next-generation sequencing (NGS) platform on bronchoalveolar lavage fluid samples from patients with severe pneumonia. This led to the identification of a *betacoronavirus* that had never been seen before. The virus was named novel Coronavirus 2019 (nCoV-2019), and the result was publicly announced on January 9, 2020 by WHO (Hu *et al.*, 2021).

Afterwards, on January 13, 2020, the Ministry of Public Health (MOH) of Thailand reported the first imported case of laboratory confirmed nCoV-2019 from Wuhan. On 15 January 2020, the Ministry of Health, Labor and Welfare of Japan (MHLW) reported an imported case of laboratory-confirmed 2019-novel coronavirus (2019-nCoV) from the same source location (WHO, 2020c). On 20 January 2020, the Republic of Korea reported the first case of novel coronavirus, and phylogenetic analyses of whole genome sequences showed that it clustered with other nCoV-2019 reported from Wuhan, China (Park *et al.*, 2020). Within one month, all 34 provinces of China reported cases of patients infected with the new virus, and the number of confirmed cases dramatically increased, with thousands of new cases reported daily during late January (R. Wang *et al.*, 2020). Finally, WHO declared the nCoV-2019 a public health emergency of international concern (PHEIC) on January 30, 2020 (WHO, 2020b). The virus was re-named “SARS-CoV-2” by the *Coronaviridae* Study

Group (CSG) of the International Committee on Taxonomy of Viruses. Indeed, based on phylogeny, and taxonomy, the CSG recognized and designated this virus as a sister clade to the severe acute respiratory syndrome coronavirus (SARS-CoV) prototypes of humans and bats linked to severe acute respiratory syndrome-like ([Coronaviridae Study Group of the International Committee on Taxonomy of, 2020](#)). WHO declared the newly named Coronavirus Disease (COVID-19) outbreak a global pandemic on March 11, 2020 as the number of the reported cases outside China increased dramatically in a short period of the time after the virus was identified ([WHO, 2020g](#)). Consequently, countries and territories worldwide have enforced lockdowns of varying levels in response to the COVID-19 global pandemic. In some countries, lockdown included total movement control while other countries have implemented restrictions based on time and disease dynamic.

3.2. Phylogeny and structure of SARS-CoV-2

According to the Baltimore classification, viruses belonging to the *Coronaviridae* family belong to class IV viruses which are single-stranded positive-sense and enveloped RNA viruses. However, following antigenically classification, SARS-CoV-2 belongs to the order of the *Nidovirales* comprising three families, the *Coronaviridae*, the *Arteriviridae*, and the *Roniviridae*. The *Coronaviridae* family is divided in two subfamilies, *Coronavirinae* and *Torovirinae*. There are four genera within the *Coronavirinae* subfamily, Alpha, Beta, Gamma, and Delta coronaviruses ([Beig Parikhani et al., 2021](#)). Within genus, viruses are grouped into sub-genus. For example, the genus Alphacoronavirus has 2 sub-genera of interest in humans, *Duvinacovirus* (species: HCoV-229E), *Setracovirus* (species: HCoV-NL63), and the genus Betacoronavirus consisting of five main sub-genera, *Embecovirus* (species: HCoV-OC43), *Merbecovirus* (species: HCoVHKU-1), *Sarbecovirus* (species: SARS-CoV and SARS-CoV-2). Other coronaviruses found in bats and that are close to human betacoronaviruses belong to the sub-genera *Nobecovirus* and *Hibecovirus*. Indeed, β -coronaviruses are known to infect wildlife, domestic animals and humans, causing unexpected outbreaks and asymptomatic infections. Although bats are the prime suspect as the source or reservoir of the virus (Figure 10), there may be intermediate hosts in the bat-to-human transmission chain (Figure 11) ([Xu et al., 2020](#)). While most scientists admit that SARS-CoV-2 is a virus of animal origin and that it would adapt to humans, they disagree on the mechanisms that led to its transition to humans, but also on the identification of the

animal virus that would be its precursor. Chun et al. have reported that SARS-CoV-2 could be a recombinant virus that is closely related to SARS bat virus, SCCoVZC21 strain (NCBI accession number MG772934) and bat-SL-Co VZC45 strain with approximately 89% sequence homology. RaTG13, bats coronavirus, has the most similarity with the novel SARS-CoV-2 (92-96% similarity) (C. Li *et al.*, 2020). However, Pekar et al., who analyzed circulating SARS-CoV-2 strains named lineages A and B before February 2020, reported that the two lineages likely resulted from two separate zoonotic transmission events from different species to humans, suggesting that evolutionary adaptation of these viruses in humans was not necessary for the spread of SARS-CoV-2 similar like SARS-CoV-1 and MERS-CoV, whose emergence was the result of repeated introduction in humans from zoonotic hosts (Pekar *et al.*, 2022).

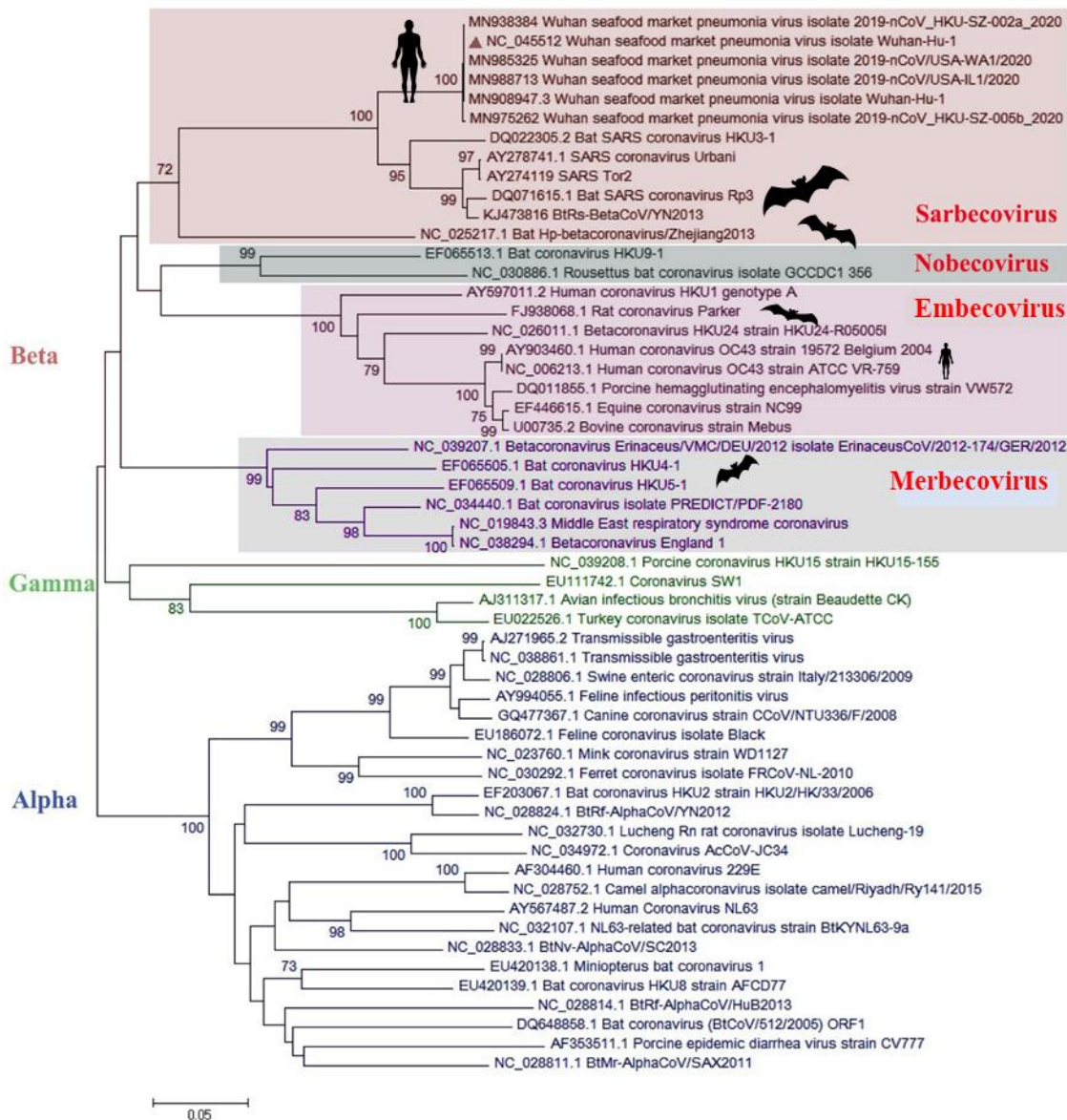


Figure 10. Phylogenetic analysis of full-length genomes of SARS-CoV-2.

The phylogenetic tree was created by analyzing 300 base regions of the coronavirus RNA-dependent RNA polymerase gene using the Neighbor Joining 1,000 bootstrap method, the red triangle showed the SARS-CoV-2 reference sequence, the scale represents the alpha lineage Coronavirus (blue font), gamma (green font) and beta (red font). The beta genus is divided into four subgenera, including Sarbecovirus, Nobecovirus, Merbecovirus, and Embecovirus. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2 (Tabibzadeh *et al.*, 2021).

In the last two decades, two highly pathogenic beta-coronaviruses of zoonotic origin have been identified: SARS-CoV-1 in civet that were probably infected by bats and MERS-CoV in camel probably infected by bats (Coleman *et al.*, 2014). Indeed, Coronaviruses are

spherical polyhedral viruses between 80 and 160 nm in diameter and have large genomes size (~27.6 to 31.6 kb) with 5'-cap structure and 3'-poly-A tail (Navas-Martin *et al.*, 2004). In electron microscopy images, Coronaviruses display club-like surface projections or peplomers composed of trimers of a spike (S) protein. The viral envelope is protected by membrane glycoprotein (M), the most common structural protein of viruses. The M protein forms viruses and promotes membrane curvature and nucleocapsid adhesion. The envelope also contains small amounts of transient membrane proteins called envelope (E) proteins, which play important roles in virus assembly, replication, and pathogenesis. The nucleocapsid (N) is another viral protein that binds to the RNA genome to form a symmetrical helical nucleocapsid (Figure 12). The nucleocapsid has two domains that can interact with the RNA genome by different mechanisms (Y. Chen *et al.*, 2020; M. Y. Wang *et al.*, 2020).

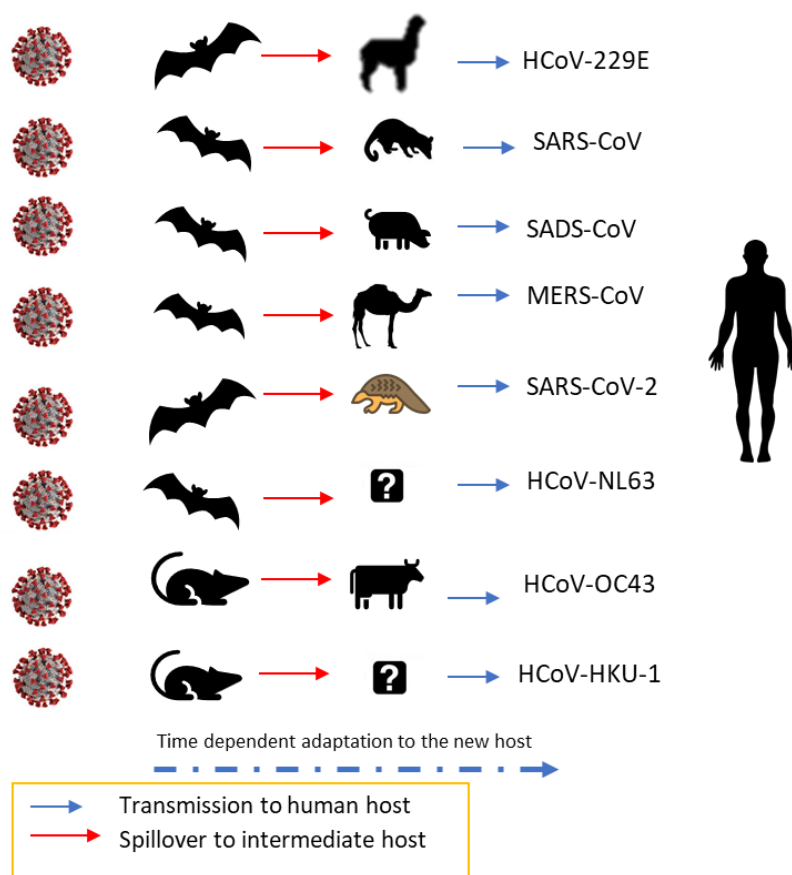


Figure 11. Animal origins of human coronaviruses (adapted from Tomasz Dzieciatkowski *et al.*, 2020).

The SARS-CoV-2 genomic RNA is used as a translation model for polyprotein 1a/1ab (pp1a/pp1ab), which encodes sixteen nonstructural proteins (nsps) important for the replication-transcription complex (RTC) in double-membrane vesicles (DMV). Thereafter, a nested set of subgenomic RNAs (sgRNAs) is synthesized by RTC in a discontinuous transcription method. These subgenomic messenger RNAs (mRNAs) have common sequences of 5' leader and 3' terminal. Termination of transcription and subsequent acquisition of a leader RNA occur at transcriptional regulatory sequences located between open reading frames (ORFs). These negative stranded sgRNAs serve as templates for subgenomic mRNA production (Y. Chen *et al.*, 2020; M. Y. Wang *et al.*, 2020).

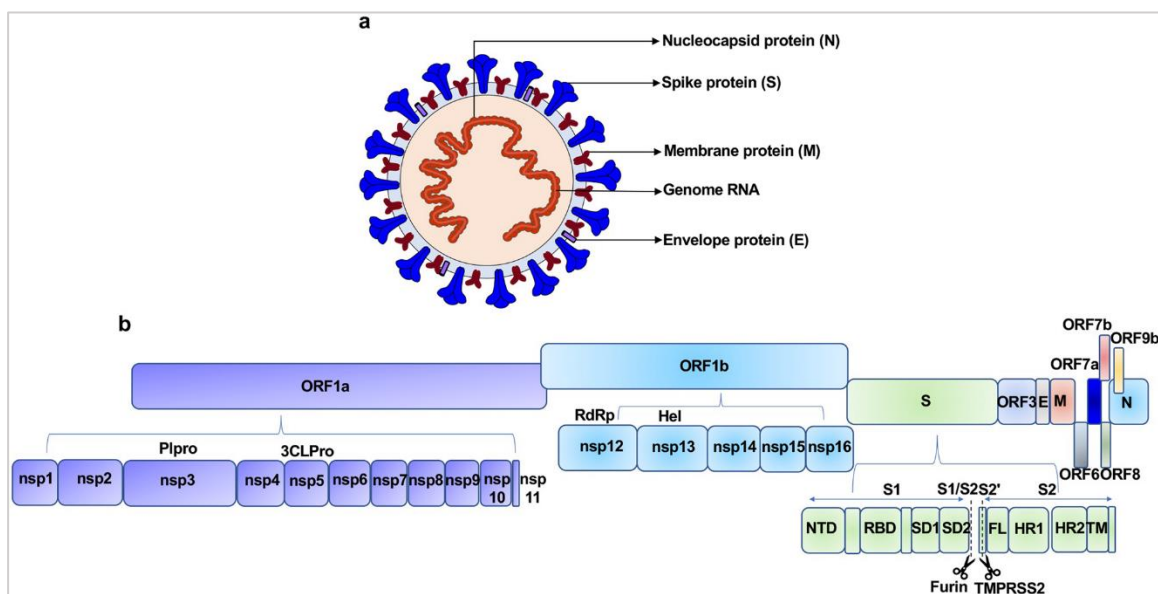


Figure 12. The structure of SARS-CoV-2 virus and molecular architecture of its genome.

a. Four structural proteins of SARS-CoV-2 include spike protein (S), membrane protein (M), nucleocapsid protein (N), and envelope protein (E). b. The genome includes ORF1a-ORF1b-S-ORF3-E-M-ORF6-ORF7(7a and 7b)-ORF8-ORF9b-N in that order. Sixteen non-structural proteins (nsp1–11, 12–16) are respectively encoded by ORF1a and ORF1b, and six accessory proteins have been described. Plpro papain-like protease, 3CLPro 3C-like proteinase, RdRp RNA-dependent RNA polymerase, Hel Helicase, S encodes N-terminal domain NTD, receptor binding domain RBD, subdomain 1 SD1, subdomain 2 SD2, fusion loop FL, heptad repeat HR1 1, HR2 heptad repeat 2, TM transmembrane domain. The dotted line indicates the cleavage of the S1/S2 and S2 sites by Furin and TMPRSS2. (Q. Zhang *et al.*, 2021).

3.3. Role and expression of viral antigens

SARS-CoV-2 genome encodes for viral structural proteins, viral nsps, and accessory proteins. Among the viral structural and accessory proteins, the S, M, and E proteins are integrated to the viral membrane to form virions. The S-trimeric protein present in the viral envelope specifically binds to the cellular receptor angiotensin-converting enzyme 2 (ACE2) and initiates the first step of viral infection as described above for viral entry into susceptible cells. Host cell TMPRSS2 functions as an S-activating protease that cleaves the S protein into two functional domains, S1 and S2. The S1 mainly helps in receptor binding while S2 gives structural support in the form of the stalk of S protein (Walls *et al.*, 2020b; Wrapp *et al.*, 2020; Yan *et al.*, 2020). The E protein forms ion channels in the viral membrane and plays an important role in pathogenicity. The N protein binds to viral genomic RNA (gRNA) and integrates gRNA into virions as ribonucleoprotein complexes (Mandala *et al.*, 2020). M protein is a transmembrane glycoprotein important for viral morphogenesis and budding through interaction with S, E and N proteins (Arya *et al.*, 2021). The number of additional proteins encoded by different coronaviruses remains controversial, as their coding potential is mainly based on bioinformatic predictions (Shang *et al.*, 2021). The function of all accessory proteins is poorly understood. However, they may be involved in host immune regulation and viral adaptation (D. X. Liu *et al.*, 2014).

Sixteen nsps are encoded by the genomic RNA, and among them nsp 11 is the smallest with 13 amino acid residues (aa), while nsp3 is the largest with 1299 aa residues (Yoshimoto, 2020). In fact, nsp play multiple roles in the cell cycle of the virus (Suryawanshi *et al.*, 2021). For example, nsp1 occupies the ribosomal mRNA binding channel to inhibit translation of host proteins (Schubert *et al.*, 2020); nsp2 binds to host prohibitins 1 and 2 and may play a role in disrupting the environment of the host cell (Yoshimoto, 2020); nsp3 is a papain-like protease for processing viral polyproteins; nsp4 and nsp6 form DMVs that is associated with replication-transcription complexes; nsp5 is a 3C-type protease for processing viral polyproteins; nsp7 and nsp8 are accessory factors of the RNA-dependent RNA polymerase (RdRP); nsp8 functions as a primase and also as a 3'-terminal RNA adenylyltransferase (TATase) activity (Tvarogova *et al.*, 2019); nsp9 is an RNA-binding protein (Littler *et al.*, 2020; Slanina *et al.*, 2021); nsp10 functioned as a cofactor of nsp14 and nsp16; nsp11 is an intrinsically disordered protein but its function does not yet

elucidated; nsp12 is an RdRP (Hillen *et al.*, 2020) and also a nucleotidyltransferase; nsp13 is a helicase; nsp14 is a 3'–5' proofreading exoribonuclease and guanosine N7 methyltransferase (N7 MTase) for RNA cap formation; nsp15 is a uridine-specific endoribonuclease and interferon antagonist; nsp16 is a ribose-2'-O-methyltransferase to cap genomic RNA (Brant *et al.*, 2021).

3.4. Replication and transcription of SARS-CoV-2

Replication of SARS-CoV-2 begins with attachment of the viral protein S to the cellular receptor for the angiotensin-converting enzyme 2 (ACE2) of the target host cell (Walls *et al.*, 2020a; Wrapp *et al.*, 2020; Yan *et al.*, 2020). In the second stage, the proteolytic cleavage of protein S caused by TMPRSS2 causes structural changes in protein S that initiate fusion between the viral and host membranes and the release of genomic RNA (gRNA) into the cytoplasm. Most mammalian cells express both ACE2 and transmembrane serine protease 2 (TMPRSS2), while these cell receptors and coreceptors are overexpressed in the lung and intestinal epithelial and endothelial cells, and therefore SARS-CoV-2 targets the respiratory system first, but many others as well vital organs (Hamming *et al.*, 2004; Hoffmann *et al.*, 2020). Cellular proteases promote gRNA uptake by N-bound viral proteins to initiate SARS-CoV-2 replication in the cytoplasm of infected cells. The positive viral genomic RNA (+gRNA) released into the cytoplasm thus serves directly as mRNA for the translation of ORF1a and ORF1b and also as template RNA for transcription of negative stranded RNA. Subsequent interactions of nsps, including viral RdRPs derived from the cleaved polyproteins ORF1a and ORF1b, result in the formation of RTCs on the matrix + gRNA for viral gRNA transcription and sgRNA synthesis in DMV induced by viral infection. Newly synthesized sgRNAs released from DMV encode viral structural and accessory proteins (Brant *et al.*, 2021; Hartenian *et al.*, 2020). Finally, a newly generated gRNA is encapsulated with N proteins, surrounded by a viral envelope and released from infected cells. In the final step, only one of the newly synthesized full-length viral +gRNA is packaged into each virion (Figure 13).

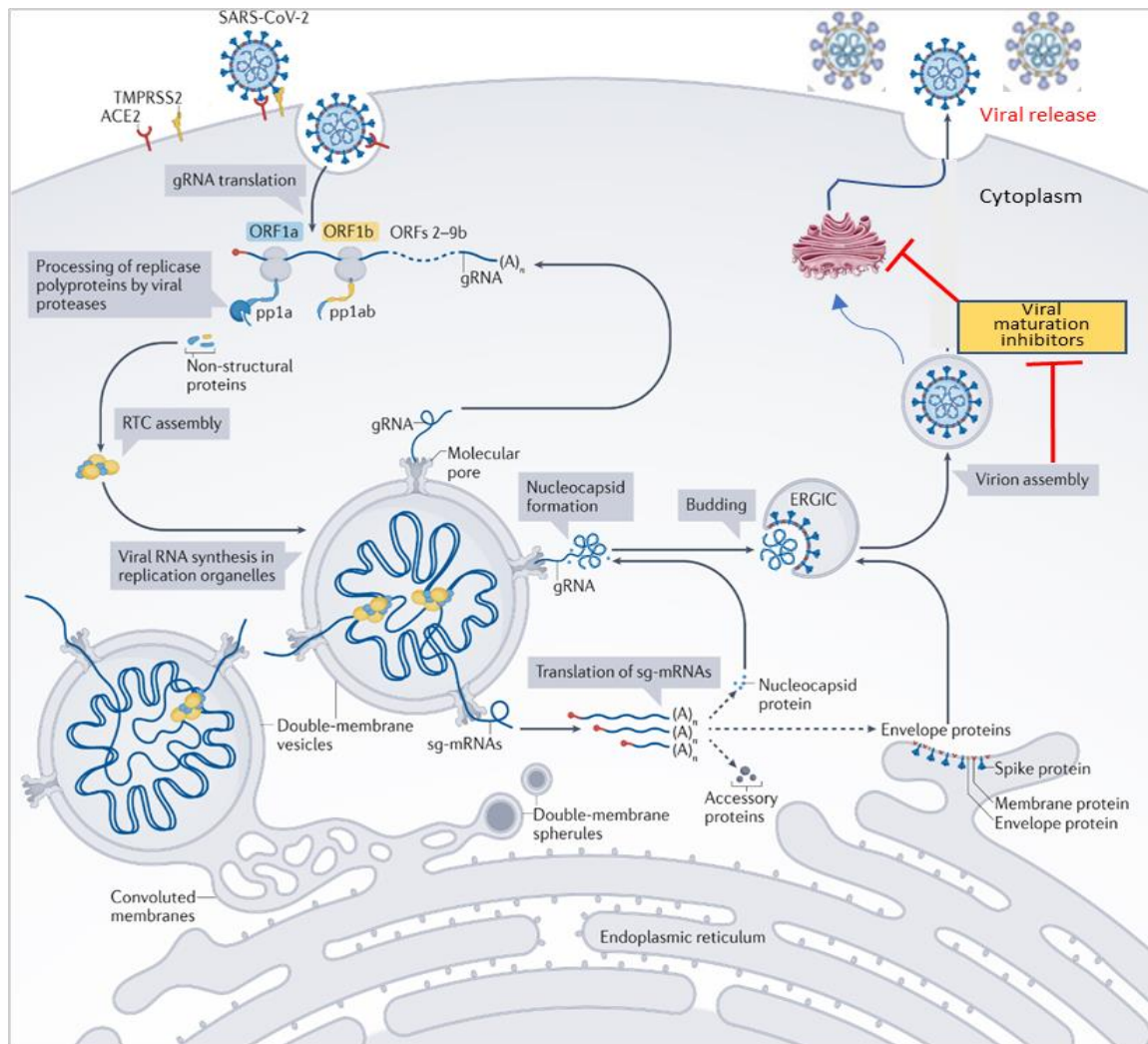


Figure 13. The lifecycle of SARS-CoV-2 in the eukaryotic cell.

The process of entry of SARS-CoV-2 into the host cell begins with the interaction of the spike protein with the cell surface protein ACE2 and is cleaved off by cellular proteases such as transmembrane serine protease 2 (TMPRSS2) to activate membrane fusion capability. Genomic RNA is released from the virus particle. Proteases incorporated into viral nonstructural protein 3 (nsp3) and nsp5 cleave pp1a and pp1ab into 16 nonstructural proteins that assemble into replication-transcription complexes (RTCs). RTCs produce novel gRNAs and a range of subgenomic mRNAs (sg-mRNAs) containing 2–9b open reading frames (ORFs) that code for structural proteins of the spike, membrane, envelope and nucleocapsid, as well as a range of accessory proteins. Newly made gRNAs can serve as a template for further RNA synthesis, or be packaged into new virions. SARS-CoV-2 assembly begins by coating gRNAs with nucleocapsid proteins, creating nucleocapsid structures that bud into the endoplasmic reticulum Golgi intermediate compartment, creating a lipid bilayer that contains the viral spike, the membrane and contains envelope proteins. Key steps inhibited by compounds under validation and which represent attractive antiviral targets are highlighted here in red. Adapted from (Malone *et al.*, 2022; V'Kovski *et al.*, 2021)

3.5. Pathogenesis of COVID-19 and Symptoms

Human infection with SARS-CoV-2 begins with the viral attachment of S1 subunit from the S protein via its receptor binding domain (RBD) to the broad receptor ACE2, which is found in a wide range of hosts. The RBD, which is the target of neutralizing antibodies and also plays a key role in viral entry, is structured by 211 aa regions (aa 319-529) at the S1-C-terminal domain of SARS-CoV-2 (Shang *et al.*, 2020; Walls *et al.*, 2020b). The receptor binding motif (RBM), which is part of the RBD, ensures contact with the ACE2 receptor. However, the SARS-CoV-2 endocytic pathway is activated by the proteolytic process. Indeed, host proteases are involved in the cleavage of protein S and then activate the entry of SARS-CoV-2, including TMPRSS2, cathepsin L and furin as previously demonstrated (Hamming *et al.*, 2004; Hoffmann *et al.*, 2020). The known tissue tropism of SARS-CoV-2 can be explained by the fact that TMPRSS2 is highly expressed in multiple body tissues and sites and is commonly co-expressed with ACE2 in nasal epithelial cells, lungs and bronchial branches (Shang *et al.*, 2020). After entering the virus in respiratory epithelial cells, SARS-CoV-2 will promote its replication and transcription, then migrate to the respiratory tract and eventually infect the alveolar epithelial cells of the lungs. Due to the rapid replication of the virus in the epithelial cells of the lungs, there is a very strong immune response as a consequence. And several studies have shown that cytokine storm syndrome is responsible for the severe respiratory distress syndrome that occurs in patients with COVID-19, which is the leading cause of death associated with the COVID-19 (Huang *et al.*, 2020; Mehta *et al.*, 2020). Similarly, histological changes following lung infection consist of bilateral diffuse alveolar damage, hyaline formation, desquamation of pneumocytes, and fibrin deposits in the lungs of patients who have developed severe COVID-19 (N. Chen *et al.*, 2020; Huang *et al.*, 2020; Martines *et al.*, 2020; Zeng *et al.*, 2020).

The incubation period of COVID-19, which is the period between exposure to the virus and the appearance of symptoms of the virus, is between 2 and 14 days with a median of 5 days. Before symptoms, infected people can be contagious and transmit the virus to healthy people. The pathogenesis of SARS-CoV-2 infection in humans can vary from mild symptoms to severe respiratory failure, and the development of clinical disease is divided into four stages: asymptomatic disease, mild disease, moderate disease or serious illness

(Hu *et al.*, 2021). Published data that described the clinical features of COVID-19 have shown that all ages of the population are susceptible to SARS-CoV-2 infection, and the median age of the infection ranges between 40 to 50 years old (N. Chen *et al.*, 2020; Huang *et al.*, 2020). Typical symptoms of COVID-19 disease are, fever, dry cough, myalgia, and fever for mild to moderate COVID-19, and dyspnea for severe cases. Less common symptoms are, sputum production, anosmia, hemoptysis, anorexia, sore throat, chest pain, chills, agnosia, nausea or vomiting, and diarrhea (N. Chen *et al.*, 2020; Huang *et al.*, 2020). COVID-19 is known to induce asymptomatic infection in many individuals, in particular in children and young adults. However, older people (> 60 years old) with co-morbidities such as diabetes mellitus, cardiovascular disease, hypertension, chronic kidney disease, and chronic lung disease are more likely to develop severe respiratory disease that requires hospitalization, and are predictor of the COVID-19 related death. Many studies have shown that hypertension alone was not independently associated with mortality (Bepouka *et al.*, 2022; N. Chen *et al.*, 2020; Guan *et al.*, 2020; Huang *et al.*, 2020; Sun *et al.*, 2021). Otherwise, pregnancy seems to not be not associated with severe COVID-19 outcome in published data but there is an isolate evidence of transplacental transmission of the SARS-CoV-2 from an infected mother to the neonate (Guan *et al.*, 2020; Vivanti *et al.*, 2020).

3.6. Immune response against SARS-CoV-2

SARS-CoV-2 virion contains structural proteins, S, E, and M in the membrane but also the genomic RNA complex with the N protein that produce a helical capsid. As described above, viral antigens such as S combining with the cell receptor ACE2 are involved in the infectivity of the host cells where the SARS-CoV-2 gRNA undergoes replication and transcription. Thus, the different steps in the viral entry and its replication present several possibilities for the innate immune system to sense viral components, and to facilitate the activation of inflammatory signaling pathways, cytokines production and cell apoptosis (Diamond *et al.*, 2022).

3.6.1. Innate immunity and SARS-CoV-2

The innate immune response is the first line of a structural immune response to a viral infection, such as that caused by SARS-CoV-2. In fact, the innate immune response

involves immune cells recognizing pathogens and releasing cytokines and chemokines. Cells that are part of the innate immune system include, macrophages, monocytes, dendritic cells, neutrophils, and innate lymphocytes (ILCs), e.g., natural killer cells (NK cells). These cells express a family of receptors called pathogen recognition receptors (PRRs), which recognize invasive components called pathogen-associated molecular patterns (PAMPs) and activate inflammatory signaling pathways and immune responses. Several PRRs and inflammasomes, such as toll-like receptor (TLR), retinoic acid-inducible gene I (RIG-I)-like receptor (RLR), and NOD-like receptor (NLR), can activate their signaling in response to SARS-CoV-2 infection ([Diamond *et al.*, 2022](#); [Kanneganti, 2020](#)).

PRR signaling activated by SARS-CoV-2 PAMPs induces the release of IFNs (IFN- β , IFN- γ) and other pro-inflammatory cytokines including IL-1 β , IL-6, TNF, IL-12, IL-17 that their expression is increased in patients with COVID-19 ([Hadjadj *et al.*, 2020](#); [Lucas *et al.*, 2020](#); [Schultze *et al.*, 2021](#)). These cytokines are important for infection clearing and also aid to maintain the cellular homeostasis. However, dysregulation in the release of these proinflammatory cytokines will result in cytokine storm which is caused by excessive production of cytokines mediated by inflammatory cells deaths (PANoptosis) ([Karki & Kanneganti, 2021](#); [Karki, Sharma, *et al.*, 2021](#)). For example, the combination of TNF and IFN- γ can lead to a cytokine storm through the synergistic induction of inflammatory cell death in the context of COVID-19 which disrupts cellular homeostasis and promotes inflammation and disease progression. Consequently, cytokine over-reporting and inflammatory cell death may explain the multi-organ damage seen in COVID-19 patients, such as lung damage associated with severe acute respiratory distress syndrome seen in severe COVID-19 patients. Likewise, structural damage to the endothelial cell membrane and subsequent vascular leakage also contribute to the development of acute distress syndrome in patients with severe COVID-19, while vascular damage is known to be the risk factors associated with hyperinflammatory shock syndromes in children with COVID-19 ([Ackermann *et al.*, 2020](#); [Belhadjer *et al.*, 2020](#); [Rowley, 2020](#)).

Excessive cytokine release during SARS-CoV-2 infection contributes to the exhaustion of germinal centers in the lymph nodes and spleen, and this may be due to lymphocyte cell death promoted by TNF and IFN- γ signaling ([Kaneko *et al.*, 2020](#)). Furthermore, TNF over-released within the germinal centers of lymphopoietic organs may limit B cells affinity

maturation, isotype switching and impair the production of mature and highly specific antibodies (Kaneko *et al.*, 2020). In fact, the studies above have shown the mechanism that leads to the cytokine storm and showed also that high levels of released cytokines are associated with the morbidity and mortality of COVID-19. However, other studies have reported different observations between cytokine levels and disease severity. For example, a meta-analysis of cytokine concentration in severe and critical COVID-19 and comparison with other inflammatory syndromes showed that the combined mean IL-6 concentration in patients with critical COVID-19 was not statistically higher than in patients with severe COVID-19. Interestingly, the aggregate mean concentration of IL-6 in patients with critical COVID-19 was significantly lower than in patients with all other non-COVID-19 comparative inflammatory diseases such as sepsis (Leisman *et al.*, 2020). Likewise, another study has reported reduced cytokine expression in peripheral blood mononuclear cells (PBMC) of COVID-19 patients than in those infected by influenza virus (Mudd *et al.*, 2020). Overall, cytokines play an important role during SARS-CoV-2 infection as part of the immune response and successful elimination of the infection. However, their release must be controlled to avoid the dramatic systemic cytokine storm that leads to pathogenic inflammation.

Innate immune responses against viral infection aim to stop viral replication but also complete infection clearance. However, some viruses such as SARS-CoV-2 have developed innate immune evasion strategies to counteract the host defense mechanisms. Among them we can cite the reduction of the IFN levels seen in COVID-19 patients with mild and moderate clinical form of the disease. Indeed, in a cohort of diagnosed COVID-19 patients in Santa Clara, researchers observed a reduction of type I and III IFNs responses to SARS-CoV-2 combining with high levels of other pro-inflammatory cytokines (Blanco-Melo *et al.*, 2020). SARS-CoV-2 viral proteins also play an important role in viral evasion to the innate immunity. For example, the SARS-CoV-2 papain-like protease (PLpro) inhibits melanoma differentiation-associated protein 5 (MDA5) activation by de-ISGylating MDA5, because ISG15 conjugation, or ISGylating, of the MDA5 CARD domain is essential for its activation following infection by RNA viruses (G. Liu *et al.*, 2021). The interaction between SARS-CoV-2 ORF9b, N and M proteins and cells receptors RIG-I and MDA5 pathways results in the inhibition of the IFN- β and pro-inflammatory cytokines expression (J. Y. Li *et al.*, 2020) while SARS-CoV-2 ORF6 and ORF8 inhibit the

expression of IFN- β and the activation of interferon-stimulated genes (ISGs) (K. Chen *et al.*, 2020; Han *et al.*, 2021; J. Y. Li *et al.*, 2020; Sui *et al.*, 2021). The knowledge gained in studying innate immune response pathways in SARS-CoV-2 infection that triggered by viral antigens evasion mechanisms can be important for the development of specific treatments and vaccines.

3.6.2. Adaptive immunity and SARS-CoV-2

The innate immune response is not sufficient for the complete elimination of SARS-CoV-2 infection, as the pathogen involves several mechanisms to evade innate responses, as explained above. Therefore, antigenic responses specifically induced via the powerful adaptive immune system are required to clear the infection. Although B and T lymphocytes are important components of adaptive cellular and humoral responses, it is clear that the innate immune system plays an important role in initiating and optimally regulating the adaptive immune response. During the internalization of the pathogen by innate immune cells such as macrophages and dendritic cells, cytokines are released to stop the infection, but also to initiate the stages of antigen presentation to the lymphocytes. Thus, activated adaptive immunity will produce robust cellular and humoral immune responses (Figure 14). In the present chapter, we are going to describe in depth the human humoral immune response against SARS-CoV-2 and its implication in the pandemic management.

The humoral immune response to SARS-CoV-2 is mediated by viral components such as the S and N proteins, but the main antigen is the S protein, which through the RBD binds the virus to the human ACE2 receptor and allows it to enter the human cell. As in all viral infections, serological studies characterizing naturally infected populations, IgM and IgA antibodies are primarily produced and contribute to antigen regulation. IgG antibodies appear later in the immune response as they change their affinity and isotype for the target antigen and increase their ability to neutralize pathogens (Van Caesele *et al.*, 2020; S. Zhang *et al.*, 2022). Several serological studies performed on patients infected with SARS-CoV-2 have reported significant heterogeneity for the correlation of antibody levels with disease severity (Gudbjartsson *et al.*, 2020; Long *et al.*, 2020; Ripperger *et al.*, 2020; Robbiani *et al.*, 2020; Roltgen *et al.*, 2020). For example, a study conducted in Wanzhou district in China showed that the virus-specific IgG levels in the asymptomatic group were

significantly lower than those detected in the symptomatic group (Long *et al.*, 2020). Otherwise, most of the individuals infected with SARS-CoV-2 are susceptible to generate antibody responses in serum and saliva (Isho *et al.*, 2020) within 2 to 4 weeks after symptom onset, and neutralizing antibodies are detected in convalescent sera from symptomatic patients and also in asymptomatic children and adults (Ripperger *et al.*, 2020; Roltgen *et al.*, 2020).

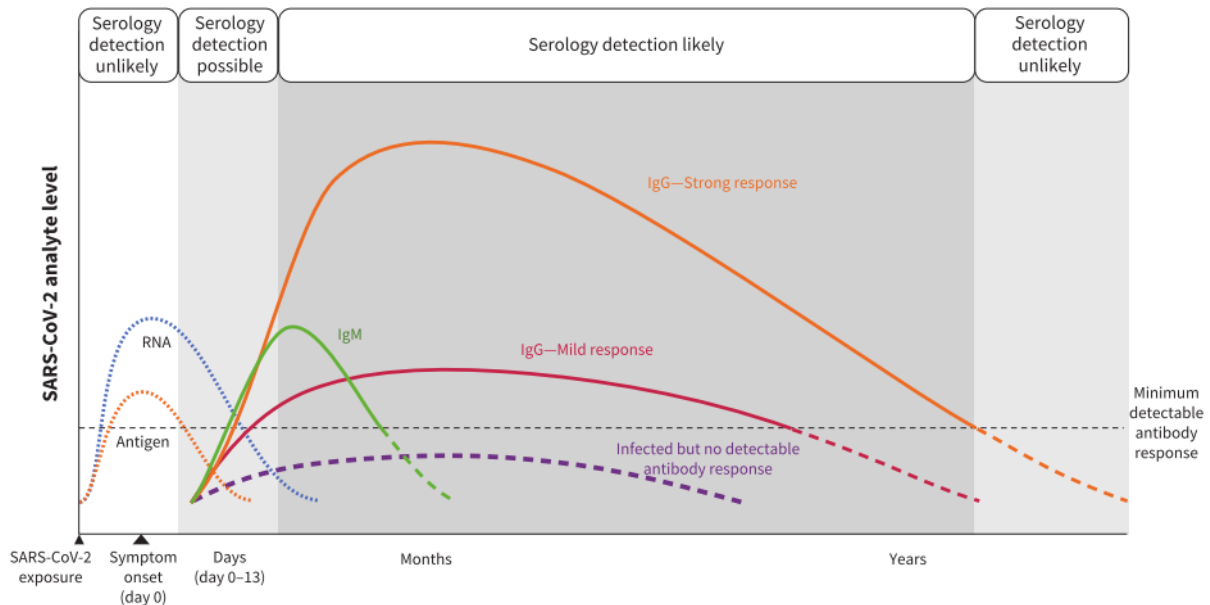


Figure 14. Description and projection for the kinetics of antibody response to infection caused by SARS-CoV-2 (Van Caesele, *et al.*, 2020).

Neutralizing antibodies to SARS-CoV-2 infection seem to be correlated with the protection from reinfection for six months following acute infection, and the probability of reinfection even during a large COVID-19 outbreak with high attack rate is lower than those naïve for SARS-CoV-2 (Addetia *et al.*, 2020; S. F. Lumley *et al.*, 2021; Pray *et al.*, 2020). Indeed, primary data reporting persistence of antibody response in COVID-19 patients alarmed the extremely rapid decline of antibodies to SARS-CoV-2, particularly in asymptomatic and milder clinical forms of COVID-19 (Long *et al.*, 2020). However, many other studies conducted subsequently estimated the durability of anti-SARS-CoV-2 antibodies at 3 to 8 months, including that of antibodies with neutralizing activities against the virus. Published studies on antibody kinetics after SARS-CoV-2 infection have reported consistent data on

the duration of anti-SARS-CoV-2 IgG, although they differ in their interpretation ([Gudbjartsson et al., 2020](#); [Isho et al., 2020](#); [Ripperger et al., 2020](#)). The decrease in antibody production after natural infection or vaccination is not linear, however it is difficult to extrapolate based on a short follow-up period of the above cited studies. In general, long-lived plasma cells that produce antibodies have a half-life of about 30 days in the first few months and may not reach a stable state for about 3 years ([Amanna et al., 2007](#); [Bortnick et al., 2012](#)). Ultimately, a study that assessed and characterized the longevity of immunity in general in different individuals to vaccines or after natural infection reported that individuals who produce higher initial antibody levels also tend to have slower decay rates and have a longer duration of protection, suggesting a significant heterogeneity in ability of individuals to produce stable humoral immune response after natural infection or vaccination ([A. Antia et al., 2018](#)).

As recommended by WHO, antibody detection has been widely used and evaluated in order to determine the extent of SARS-CoV-2 infection in populations worldwide ([WHO, 2020e](#)). Therefore, the accuracy of several reported SARS-CoV-2 seroprevalences depends on the accuracy and performance of the diagnostic tools used for antibody detection, which rely primarily on recombinant proteins. Therefore, to eliminate the bias that could explain the overestimation of SARS-CoV-2 seroprevalence, scientists evaluated the potential cross-reactivity between SARS-CoV-2 and other alpha and beta coronaviruses using ELISA. Among them, Jennifer H. et al. reported serological cross-reactivity between endemic and seasonal coronaviruses. In fact, they analyzed the potential serological cross-reactivity of the SARS-CoV-2 S protein, which also correlates with neutralizing antibodies, with the MERS-CoV and SARS-CoV-1 S protein previously implicated in major outbreaks, and with other seasonal beta-coronaviruses (HCoV-OC43, HCoV-HKU-1), and alpha-coronaviruses (HCoV-229E, HCoV-NL43) S proteins. Consequently, antibodies directed against HCoV-OC43 and HCoV-HKU1 have minimal cross-reactivity with the SARS-CoV-2 pandemic but also with the other two coronaviruses, MERS-CoV and SARS-CoV. This can be explained by the fact that the SARS-CoV-2 protein S sequence is more similar to SARS-CoV-1 and MERS-CoV, in contrast to the seasonal coronaviruses HCoV-OC43 and HCoV-HKU1 ([Hicks et al., 2021](#)). Although antibodies against S protein of pandemic SARS-CoV-2 likely cross-reacted with MERS-CoV and SARS-CoV S proteins, cross-

neutralization of the live viruses seems to be rare, suggesting the presence of a non-neutralizing antibody response to conserved epitopes in the S protein.

3.7. Transmission and epidemiology of SARS-CoV-2

3.7.1. SARS-CoV-2 Transmission

As previously described, coronaviruses are of zoonotic origin. Therefore, several factors including, the viral low fidelity of RdRP, wide distribution of the human receptor ACE2, and the environmental and socio-economic factors such as domestication of animals, urbanization and commercial trade increase the risk for transmission of zoonotic pathogen of animals to humans and vice versa (P. G. da Silva *et al.*, 2021). In fact, routes of the transmission for SARS-CoV-2 include physical contact, droplet, airborne, fomite, fecal-oral, bloodborne, mother-to-child, and animal-to-human transmission.

Airborne transmission. The transmission of SARS-CoV-2 among humans is caused by the dissemination of droplet nuclei called “aerosols” remaining infectious for up to 16 hours when suspended in air (WHO, 2020f). Airborne transmission of SARS-CoV-2 can occur during medical procedures that generate aerosols, normal breathing and speaking can lead to exhaled aerosols, and respiratory droplets also generate microscopic aerosols (<5 µm) through evaporation as well (Asadi *et al.*, 2020; Bourouiba, 2020; Morawska *et al.*, 2020). However, studies that have evaluated the presence of the virus in the air via aerosols and its contagiousness are controversial. Van Doremalen *et al.* evaluated the surface stability of SARS-CoV-2 aerosols in an experimental study. They found SARS-CoV-2 virus RNA in aerosol air samples for up to 3 hours, while Fears A. *et al.* reported the presence of viruses in aerosols 16 hours after aerosol generation (Fears *et al.*, 2020; van Doremalen *et al.*, 2020). Similarly, studies conducted in healthcare facilities caring for symptomatic patients with COVID-19 but not using aerosol-generating procedures have reported the presence of the virus in air samples (Chia *et al.*, 2020; Guo *et al.*, 2020), and conversely, other similar studies found no presence of SARS-CoV-2 RNA in air samples (Cheng *et al.*, 2020; Faridi *et al.*, 2020; Ong *et al.*, 2020). Additionally, no studies have found viable viruses in air samples (Cheng *et al.*, 2020; Faridi *et al.*, 2020; Ong *et al.*, 2020). Ultimately, it remains important to continue studies on the aerosol transmission of SARS-CoV-2 outside medical

structures that generate aerosols. These include closed and populated spaces, restaurants and churches where studies suspect a high possibility of transmission of the virus through aerosol emissions.

Droplet transmission and contact. SARS-CoV-2 can be transmitted through direct, indirect, or close contact with an infected person through infected secretions, such as saliva, respiratory secretions, or respiratory droplets when an infected person coughs, sneezes, talks, or sings. Respiratory droplets are 5-10 μm in diameter and can be transmitted by close contact (within 1 meter) of an infected person who has respiratory symptoms or who is talking or singing. In these cases, respiratory droplets containing the virus can reach the mouth, nose or eyes of a susceptible person and cause infection ([WHO, 2020f](#)).

Fomite transmission. Surfaces and inanimate objects contaminated with droplets or respiratory secretions expelled by COVID-19 patients can transmit infection. Indeed, SARS-CoV-2 viral RNA has been detected in numerous published reports ([Chia et al., 2020](#); [Ong et al., 2020](#)). Transmission can occur in health facilities caring for COVID-19 patients, but also by touching surfaces in the immediate vicinity of the infected person, or by touching objects contaminated with the virus and then touching their nose, mouth, or eyes. Although there is evidence of SARS-CoV-2 contamination of surfaces and objects, no specific reports have directly demonstrated carrier transmission.

Additionally, SARS-CoV-2 can be transmitted through other biological fluids. RNA of the virus has been detected in urine and feces, suggestion the possibility of the transmission by these routes. Other studies have revealed the presence of the virus in the plasma or serum, and the evidence of the replication of the virus within blood cells, but there is no evidence of bloodborne transmission to date. Furthermore, we previously mentioned the study that reported one case of mother-to-child transmission via placenta but data remain extremely limited. Although the virus can be found in breast milk, studies that have investigated did not found viable virus.

3.7.2. Epidemiology of SARS-CoV-2

Since COVID-19 was announced as a global pandemic on March 11, 2020, the virus has been rapidly spreading, affecting communities from all over the world. On August 2, 2022,

a total of 575,887,049 infections with 6,398,412 of deaths have been reported in 214 countries and territories (WHO, 2022e). Europe, America and Asia are the regions where COVID-19 cases are most reported. The COVID-19 pandemic is characterized by the worldwide spread of the SARS-CoV-2 virus in the form of epidemic waves. Indeed, since the declaration of the pandemic, six epidemic waves of COVID-19 have been reported worldwide, but with specificities linked to each continent and country (Figure 15). Many factors can explain the observed heterogeneity in the distribution of SARS-CoV-2 around the world. For example, in several countries, the authorities have introduced restrictive measures such as national lockdown, the suppression of international trade and flights, the limitation of gatherings and the obligation to wear a mask. These measures may have slowed the spread of the virus in some areas. Other policies that may have had an impact on the spread of the pandemic are the disparities found in many countries in the use of vaccines as a preventive method. Indeed, vaccines could increase the number of protected people and help stop the spread of the virus in some countries.

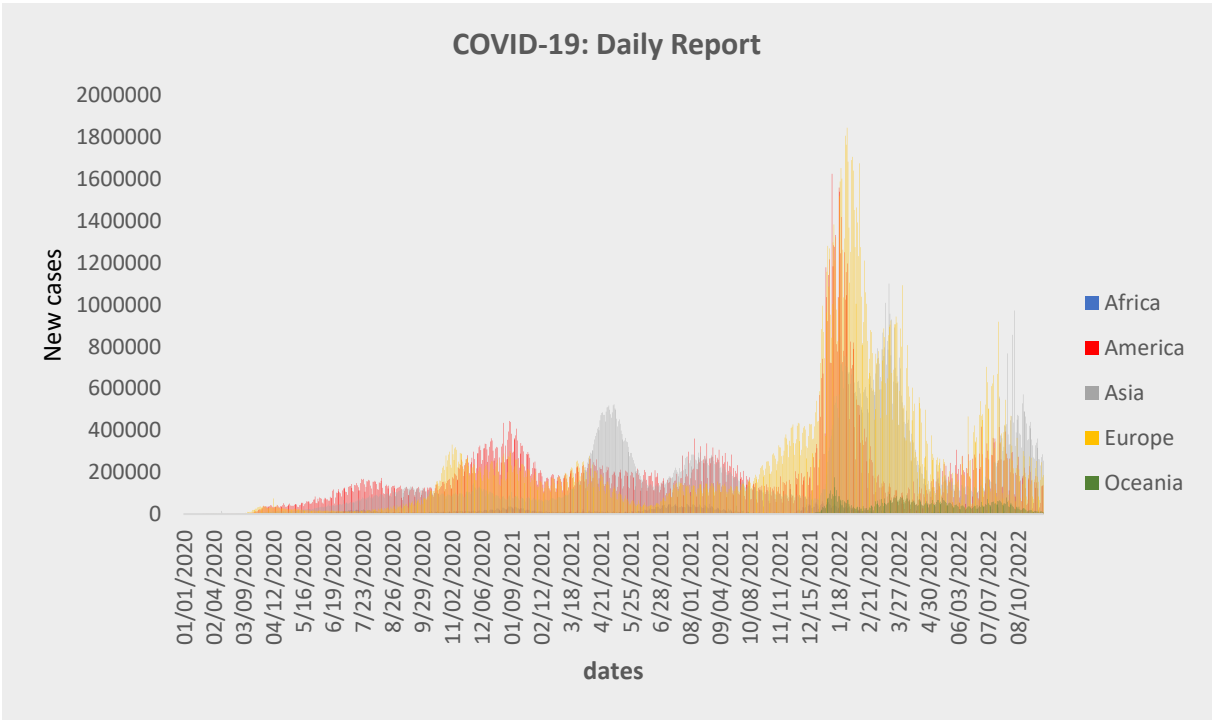


Figure 15. Global situation of reported confirmed COVID-19 cases during the pandemic (WHO, 2022b).

Several other factors seem to explain the difference in the prevalence of COVID-19 in different regions of the world. Some authors have incriminated the role of meteorological factors in the transmission of the virus. In fact, the optimal average temperature is between 5 and 11°C ([Audi *et al.*, 2020](#); [Moriyama *et al.*, 2020](#)). Additionally, some people infected with SARS-CoV-2 remain asymptomatic, and as previously mentioned, the asymptomatic form is more common in children and young people. Thus, in a region dominated by a young population, the reported prevalence of COVID-19 disease based on RT-PCR results may be falsely low. It is therefore useful to complete these results with seroprevalence studies.

3.7.3. Epidemiology of SARS-CoV-2 variants

Most of the RNA viruses including SARS-CoV-2 are likely to be susceptible to undergo several mutations. Usually, mutations do not affect the virus properties, resulting in what is called “silent mutation”. However, some changes may make the virus more transmissible, more virulent, resistant to therapeutic medicines, or reduce the efficacy of the vaccines. Thus, the molecular evolution of SARS-CoV-2 has been assessed since January 2020 in order to characterize the circulating variants of interest (VOI) or variants of concern (VOC). Indeed, a variant is considered as VOC when it had increased transmissibility or changed the disease epidemiology, increased in virulence or lead to change clinical disease presentation, or decreased the effectiveness of public health and social measures or available diagnostics, vaccines, or therapeutics. The activity that consist in assessment of VOC is defined by WHO, expert networks, national institutions and independent research groups. The Table 3 gives the history of VOCs reported by WHO since 2020 ([WHO, 2022d](#)).

Table 3. Previously and currently circulating variants of concern.

WHO label	Pango lineage	GISAIID clade	Nextstrain clade	Earliest documented samples	Date of designation
Alpha	B.1.1.7	GRY	20I (V1)	United Kingdom, Sep-2020	VOC: 18-Dec-2020
Beta	B.1.351	GH/501Y.V2	20H (V2)	South Africa, May-2020	VOC: 18-Dec-2020
Gamma	P.1	GR/501Y.V3	20J (V3)	Brazil, Nov-2020	VOC: 11-Jan-2021
Delta	B.1.617.2	G/478K.V1	21A, 21I, 21J	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021
Omicron*	B.1.1.529	GR/484A	21K, 21L, 21M, 22A, 22B, 22C, 22D	Multiple countries, Nov-2021	VUM: 24-Nov-2021 VOC: 26-Nov-2021
Omicron	BA.1 + BA.1. **		21K (Omicron)	Southern Africa	November 2021
Omicron	BA.2 + BA.2**		21L (Omicron)	Southern Africa	November 2021
Omicron	BA.2.12.1		22C (Omicron)	Canada/USA	December 2021
Omicron	BA.2.75**	GRA	22D (Omicron)	India	June 2022
Omicron	BA.3		21K (Omicron)	Southern Africa	November 2021
Omicron	BA.4**		22A (Omicron)	Southern Africa	January 2022
Omicron	BA.5**	GRA	22B (Omicron)	Southern Africa	January 2022
Omicron	BA.4.6	GRA	22A (Omicron)	USA	April 2022
Omicron	BQ.1		22E (Omicron)	Nigeria	July 2022
Omicron	BQ.1.1		22E (Omicron)	Nigeria	July 2022
Omicron	XBB		22F (Omicron)	USA/Singapore	August 2022
Omicron	XBB.1		22F (Omicron)	USA/Singapore	September 2022
Omicron	BF.7		22B (Omicron)	Multiple	June 2022

* Includes BA.1, BA.2, BA.3, BA.4, BA.5 and descendent lineages. It also includes BA.1/BA.2 circulating recombinant forms such as XE.

** Denotes mutations found in at least >25% variants, but not in all sequences.

- VOI: variants of interest; VOC: variants of concern; VUM: variant under monitoring.

3.8. Diagnosis

In the 21st century, there are very clear technological achievements, especially in medicine. The knowledge gained in the context of other viral pathogens was used to develop reliable methods for the detection of SARS-CoV-2 in human samples in a very short time. In fact, rapid and accurate diagnostic testing is essential for the identification and management of infected individuals, contact tracing, epidemiologic characterization, and medical decision making. In addition to clinical diagnosis based on symptoms, non-specific laboratory markers, and medical imaging, several specific laboratory diagnostic methods have been developed for the detection of SARS-CoV-2, including in vitro diagnostics using molecular methods and serological tests for antibodies and antigen-based tests.

Molecular test, nucleic acid amplification test (NAAT). SARS-CoV-2 infection is confirmed by detection of SARS-CoV-2 RNA using NAAT that is considered as gold standard, and SARS-CoV-2 NAATs are performed on upper respiratory samples using swabs obtained from nasopharynx, oropharynx, nasal mid-turbinate, or anterior nares (Alsuliman *et al.*, 2020). RT-PCR is recommended as the most sensitive NAAT method for the detection of an RNA virus such as SARS-CoV-2. Indeed, conventional NAAT, also known as a two-step test, usually takes 3.5-4.0 hours and starts with RNA extraction from respiratory samples followed by RT-PCR in which purified total RNA is first reverse transcribed in complementary deoxyribonucleic acid (cDNA) by reverse transcriptase, the cDNA is then subjected to quantitative PCR (qPCR) to exponentially amplify the gene of interest (Weissleder *et al.*, 2020). Due to the universal need for RNA extraction kits during the outbreak, simplified NAATs are being developed by eliminating the RNA extraction step (Afzal, 2020; Premraj *et al.*, 2020; Weissleder *et al.*, 2020). Preliminary results from one-step RT-PCR, where RNA extraction was suppressed by simple directly heating samples at 98°C for 5 minutes, showed sensitivity and specificity to be comparable to standard methods (S. J. R. da Silva *et al.*, 2020; Fomsgaard *et al.*, 2020).

Point of care (PoC) molecular diagnostic tests. Based on epidemiological and clinical data, SARS-CoV-2 was classified as a risk group 3 organism. Therefore, molecular detection using the RT-PCR method requires the presence of qualified personnel, specialized equipment and a good biological waste management. However, the very large number of

samples to be analyzed in the context of the pandemic has highlighted the need for reliable and rapid diagnostic methods that can be implemented with limited resources in remote regions. Therefore, the development of molecular point-of-care diagnostic tests has made it possible to overcome this limitation. First, the Xpert[®] Xpress SARS-CoV-2 test (Cepheid, Sunnyvale, CA), developed and used for tuberculosis, has been widely implemented and provides qualitative virus detection in approximately 45 minutes using the GeneXpert benchtop model. GeneXpert for SARS-CoV-2 targeting N2 and E genes showed 100% concordance with conventional RT-PCR and is capable of detecting a lower limit of 8.26 copies/mL (Wolters *et al.*, 2020). A second molecular PoC is ID Now COVID-19 test (Abbott Diagnostics Scarborough, Inc., ME), which detects qualitative SARS-CoV-2 RNA from upper respiratory tract samples. ID Now COVID-19 uses an Isothermal Nucleic Acid Amplification Test (INAAT) based on Nicking Enzyme-Assisted Reaction (NEAR) technology (A. S. James *et al.*, 2020) to amplify the RdRp gene in 5-13 minutes, with a low detection limit of 125 genome equivalents/ml with a sensitivity of 80.4% and a specificity of 95.9% in clinical evaluation (Basu *et al.*, 2020; Hogan *et al.*, 2020).

End-point RT-PCR. This molecular method is based on the detection of the accumulated PCR product at the end of the amplification reaction, unlike real-time PCR, which calculates Ct values over the exponential phase of the amplification. Several endpoint methods have been developed for the detection of SARS-CoV-2, including lateral flow, fluorescence, enzyme colorimetry, electrochemistry, and matrix-assisted laser desorption ionization time-of-flight (MALDI -TOF) (C. Y. Yu *et al.*, 2021).

SARS-CoV-2 Antigen-based test. The SARS-CoV-2 antigen test is a serological test that can be used as a potential POC alternative to increasingly expensive molecular biology methods. Antigen-based diagnostics detect viral protein fragments instead of viral nucleic acids in nasopharyngeal swab specimens (Habli *et al.*, 2020). One of the advantages of this test is that it can be performed immediately after sampling and within 15 minutes. Therefore, a highly sensitive method that directly detects viral antigens in clinical specimens would be of great benefit for the rapid implementation of public health measures to reduce transmission (Lambert-Niclot *et al.*, 2020). Viral proteins are detected using antigen capture methods commonly used for other viral tests, such as human immunodeficiency virus (HIV) and hepatitis virus (Smithgall *et al.*, 2020). Based on

previous experience with antigen testing in SARS and MERS, protein N is considered an excellent target for a diagnostic test for antigen capture. Indeed, protein N is abundantly secreted during replication and exhibits low cross-reactivity with other human coronaviruses in comparison with S protein (Y. Chen *et al.*, 2015). Several publications on the validation of the antigen kit against the standard, in particular RT-PCR using swabs, have reported variable performances of antigenic tests. Lambert-Niclot *et al.*, have evaluated a rapid test named COVID-19 Ag Respi-Strip (Coris Bio-Concept, Gembloux, Belgium), and they reported a specificity of 100% and sensitivity of 50% compare to the RT-PCR results (Lambert-Niclot *et al.*, 2020). In another evaluation of the same antigenic test, reported sensitivity and specificity were 57.6% and 99.5% respectively (Mertens *et al.*, 2020). Scohy *et al.*, however, observed that the overall sensitivity of COVID-19 Ag Respi-Strip test was around 30% compare to the RT-PCR results (Scohy *et al.*, 2020). Detection performance of lateral flow test using for detection of SARS-CoV-2 seems to be correlated to the viral load, suggesting that sensitivity decrease when Ct values increase (Corman *et al.*, 2021). Antigen POC tests can therefore be used in efforts to limit the spread of SARS-CoV-2, but due to their lower sensitivity, they might not be able to exclude SARS-CoV-2 infection.

Diagnosis based on SARS-CoV-2 antibodies. The detection of specific antibodies against SARS-CoV-2 is a technique that allows to evaluate recent or previous exposure to the virus and thus deduce the residual immunity that the individual has developed against the virus. This method holds its place among the diagnostic tools used in the context of the COVID-19 pandemic. Indeed, antibody serology is particularly useful for patients who show symptoms late after exposure to the virus, generally at least 2 weeks after the exposure (Abbasi, 2020), but also for patients who are false negatives on molecular analyzes (Yong *et al.*, 2020). Serological data are particularly useful for epidemiological purposes, such as estimating the attack rate, R_0 and mortality rate (Winter *et al.*, 2020), and for evaluating the impact of control measures such as country lockdown, obligation of wearing mask, and trade and travel restriction. Likewise, the antibody assessment may also aid in the identification of plasma donors and in evaluating the immunogenicity of the vaccine (Abbasi, 2020; Nnaji *et al.*, 2020; Winter *et al.*, 2020). However, in a pandemic environment where early diagnosis is essential for patient management and epidemic control (Ali *et al.*, 2020), antibody tests are not optimal due to delayed seroconversion and variability in

performance, and therefore are not the preferred first-line test (Lisboa Bastos *et al.*, 2020). Currently commercialized platforms for serological evaluation of antibodies include lateral flow immunoassays (LFIA), ELISA and chemiluminescence immunoassays (CLIA). These assays are based on similar principles, but differ in the method of detecting antibody-antigen binding (Kontou *et al.*, 2020). Several studies have evaluated the above described serological methods, and the results are summarized in reviews and meta-analysis. Sensitivities and specificities reported from these studies seem to be variables. However, it appears that LFIAs have lower sensitivities, but comparable specificities to ELISAs and CLIAs (Gong *et al.*, 2021; Mardian *et al.*, 2021).

3.9. Treatment

COVID-19 treatments include, non-medical preventive measures, vaccination, specific molecules, and vital supportive treatment.

3.9.1. Prevention treatment

Since COVID-19 was declared as pandemic, several countries declared country lockdown and installed restrictions. Of these, suppression of international trade and travels, observation of physical distance, obligation of wearing mask in public area, suppression of gathering, ventilation of closed area, washing hands with soap and clean water, and the use of hydroalcoholic gel have been implemented. In addition to these methods, several vaccines have been used, and others are under development in order to prevent infection and/or to protect against developing severe COVID-19 disease. Due to the pandemic situation, the development of COVID-19 vaccines has been accelerated, and vaccines received emergency use listing by the WHO, and have gone through the standard preclinical and clinical stages of development. Furthermore, vaccines have been developed following traditional approach as well as new methods of development. Some platforms develop vaccines using inactivated virus or live attenuated viruses, which have been used for inactivated influenza vaccines and measles vaccine, respectively. Other approaches employ newer platforms, such as recombinant proteins (used for human papillomavirus vaccines) and vectors (used for Ebola vaccines). Some other platforms, developed RNA and DNA

vaccines that were not previously employed in a licensed vaccine (CDC, 2022a; NIH, 2022).

In particular, the COVID-19 pandemic has seen the acceleration of the vaccine manufacturing process, particularly those that have led to new vaccines called messenger RNA (mRNA) vaccines. In fact, after the discovery of RNA in 1961 by François Gros, several experiments with RNA arose (Institut Pasteur, 2021). Around 1978, scientists used liposomes to transport mRNA in mice (Dimitriadis, 1978) and human cells (Ostro *et al.*, 1978). The fusion of the mRNA-containing liposomes with the cell membrane allowed the release of the genetic material into the cell. Later, in 1978, Krieg, Douglas Melton, Tom Maniatis and Michael Green of Harvard University had developed a new method of synthesizing mRNA using the enzyme RNA polymerase in the bacteriophage vector SP6 (Melton *et al.*, 1984). The mRNA synthesized and injected into frog eggs induced the production of proteins showing that it was functional (Krieg *et al.*, 1984). It was in 1987 that Melton *et al.* suggested that mRNA could be used to treat specific diseases or prevent others by producing antibodies, especially since mRNA could inhibit or activate protein production (Melton *et al.*, 1984). Between 1990 and 2000, the idea of using mRNA in therapy was widely adopted by many scientists, including oncologists. It was suggested that mRNA that encodes for cancer proteins could be injected in harvested immunological cells, and then injected back into the sick individuals in order to treat cancer (Boczkowski *et al.*, 1996). Although this method had shown promise in mice, large randomized trials failed to produce conclusive results (Elie Dolgin, 2021). In early 1990, Zhou *et al.* evaluated the efficacy of the recombinant RNA of the Semliki Forest virus (SFV) in eliciting immune response in mice. Using suicide particles and naked RNA for mice immunization, they reported high levels of IgG at day 30 and have observed that the antibody concentrations remained higher for up to 4 months suggesting that RNA could be used as vaccine (Zhou *et al.*, 1994).

The major progress on development of mRNA vaccine began around the 2000s when it has been suggested that direct mRNA infection of mice could induce the production of the immune response, suggesting that messenger mRNA could be used as a vaccine against many diseases. However, in order to be administered without risk of rejection by the body, the mRNA of Kariko *et al.* had proposed to rearrange the nucleotides of the mRNA, uridine,

to create "pseudouridine" (Kariko *et al.*, 2005). These recent advances contributed to the development of mRNA vaccines as we know them today. Moderna® had developed the first mRNA vaccine in lipid nanoparticles and successfully tested it in mice. Additional clinical trials of mRNA vaccines were conducted between 2010 and 2015, and by 2020, mRNA vaccines for COVID-19 had received emergency marketing authorization.

3.9.2. Curative and supportive care

The clinical treatment in patients with COVID-19 is managed following the two processes that drive the pathogenesis of SARS-CoV-2. Indeed, the disease is primarily driven by the high rate of viral replication, and later in the clinical course, the disease appears to be driven by a dysregulation of the immune response to the virus that leads to tissue damage. Thus, in the early stage of the clinical disease, medicines that target directly the virus are preferred while immunosuppressive and anti-inflammatory treatments are likely to be more efficacies in the later stages of the disease.

Non-hospitalized patients with acute COVID-19. These patients should receive supportive care, but also COVID-19 specific medicines for patients who have a high risk for disease progression (elders with comorbidities, immunocompromised patients). However, patients with dyspnea should be referred for an in-person evaluation by a health care provider and should be followed closely during the initial days after the onset of dyspnea to assess for worsening respiratory status.

Hospitalized patients with COVID-19. Patients who have developed severe or critical COVID-19 clinical form must be hospitalized in prevision of the need of supplemental oxygen. Depending on disease severity, patients hospitalized but who do not require supplemental oxygen, receive dexamethasone or other corticoid, and a prophylactic dose of heparin (anticoagulation therapy). Hospitalized patients who require supplemental oxygen will receive antiviral treatment (Remdesivir) alone, or with dexamethasone, and a therapeutic dose of heparin. For those who are hospitalized and require oxygen through a high-flow device, they should be treated with dexamethasone alone, or with remdesivir, and a prophylactic dose of heparin.

Other antivirals that can be used in children aged 12 and older are: Nirmatrelvir with Ritonavir (Paxlovid). The Molnupiravir (Lagevrio) is an antiviral that is used only in adults with COVID-19. In addition, SARS-CoV-2 monoclonal antibodies such as Bebtelovimab, Etesevimab, Casirivimab, Imdevimab, and Sotrovimab are used in combination for patients with mild to moderate COVID-19 who are hospitalized for a reason other than COVID-19. These monoclonal antibodies are administered if the patient meet the FDA emergency use authorization criteria for outpatient treatment ([CDC, 2022a](#); [NIH, 2022](#)).

3.10. Seroprevalence of SARS-CoV-2 in Africa

COVID-19 disease has been characterized by a rapid progression of infection around the world since the disease was declared a global pandemic on March 11, 2020. Several mathematical models have predicted the burden of the disease in the world but also in Africa. For example, Walker et al., suggested that 70 million Africans could be infected by SARS-CoV-2, with more than 3 million deaths ([P. G. T. Walker et al., 2020](#)). Furthermore, Wells et al., estimated that there would be 76,213,155 infections and 319,441 deaths in the absence of physical distancing and any public health measures in the DRC during the first year of the circulation of the virus ([C. R. Wells et al., 2020](#)). At the moment we started the present thesis project, the reported number of SARS-CoV-2 infections in Africa were lower than the predicted ones, suggesting a heterogeneity in the epidemic. Therefore, WHO advised to African countries as well to conduct population-based serosurveys in order to understand the real extent of the SARS-CoV-2 infection in African settings, and to adapt public health policies and measures for an efficient management of the pandemic. However, only a few studies have evaluated the SARS-CoV-2 seroprevalence in Africa, and most of these studies conducted at that time, reported seroprevalence only in specific populations limiting to generalize the results to the entire population. Additionally, it would also be very important to develop sensitive but also very specific serological tests to reduce overestimation biases linked to possible cross-reactions or those linked to the low sensitivity of the tests in relation to the types of population to be studied.

Thesis Work

Chapter 1. Context and thesis objectives

1.1. Thesis context

The DRC is a Central African country with surface of 2,345,499 square kilometers (km²) and a population of 92 million individuals, making it the second largest country in Africa. The main topography of the country includes a large river basin, large valleys, high plateaus, three mountain ranges, and low coastal plains. Most of the Congo is located in a humid inland tropical or equatorial zone, which extends from the equator to 5 degrees from north to south. Southern and northern Congo have a fairly dry sub-equatorial climate. The life of plants is very rich and depends on the climate. The heart of the Congo Basin is covered by a complex forest system commonly known as the equatorial rainforest. Animal life is also rich and diverse, e.g., non-human primates, and other mammals such as bats, rodents, felines and elephants. Birdlife includes several types of pelicans, parrots, sunbirds, pigeons, ducks, geese, eagles, cuckoos, owls, cranes, storks and swallows ([Encyclopedia Britannica, 2022](#)).

The diversity of its ecosystem and its climate make the DRC a country with a high potential for the emergence of zoonotic infectious diseases. In its modern history, the DRC has experienced multiple outbreaks of infectious diseases caused by parasites, bacteria and viruses that have caused multiple deaths and, in particular, have had major socio-economic impacts. Of these emergences, EVD, which first emerged in two simultaneous outbreaks in Nzara, Sudan, and Yambuku, DRC, is the deadliest, with a mortality rate reaching 90%. Indeed, this disease poses a major public health problem in sub-Saharan Africa regardless the recent reported events. As a zoonotic disease, the EVD index case most likely becomes infected through contact with infected animals (usually during slaughter, preparation or consumption) and subsequent human-to-human infections are generally caused by contact with biological fluids from infected humans. After an incubation period of up to 21 days, the disease initially manifests itself with a so-called "dry" phase characterized by a sudden onset of fever, intense weakness, muscle pain, headache and sore throat. Then follows the so-called "wet" or "humid" phase characterized by vomiting and diarrhea, often associated with skin rashes, impaired renal and hepatic function, and in some cases internal and external bleeding. Since the discovery of the Ebola virus, many outbreaks (n=41) have been

reported in Africa, including 15 episodes in DRC. There are 6 different viral lineages, 4 of which are responsible for human infections: *Zaire ebolavirus*, *Bundibugyo ebolavirus*, *Sudan ebolavirus*, and *Tai-forest ebolavirus*. No human infections have been reported for the other 2, *Reston ebolavirus*, which only affects non-human primates in Asia, and *Bombali ebolavirus*, recently described in insectivorous bats in Bombali district, Sierra Leone, but also in Kenya and Guinea (CDC, 2021a, 2022b).

Most EVD outbreaks have occurred primarily in Central Africa, with a limited number of victims in remote areas. However, between 2014 and 2016, EVD emerged in cities in three West African countries, affecting more than 28,000 people and killing more than 11,000. The frequency of outbreaks has increased in recent years, including in the DRC, where 6 consecutive epidemics were recorded in Likati (northeast), Equateur (northwest) and in the provinces of North Kivu, South Kivu and Ituri (northeast) between 2017 and 2022. The 10th EVD outbreak in North Kivu, South Kivu and Ituri was the largest and most difficult to control ever recorded in the DRC, resulting in 3,470 cases and 2,287 deaths. The reluctance of the population to turn to Ebola Treatment Centers (CTEs) and violence against response teams and facilities have hampered the smooth running of fieldwork and facilitated the spread of the disease. Given the risk of regional spread, this epidemic was declared a public health emergency of international concern by the WHO on July 17, 2019. Consequently, the coordination of the response was carried out under the responsibility of the Technical Secretariat of the Multisectoral Response Committee to the EVD Epidemic, in collaboration with local and international stakeholders and partners.

This thesis project initially focused on understanding the immune response against the Ebola virus in humans. However, the declaration of COVID-19 as a global pandemic by the WHO in March 2020 and the subsequent restrictions, particularly related to international travel, did not allow us to carry out our various research studies of our thesis as planned. However, we had viewed the emergence of COVID-19, caused by a newly identified virus, as a new research opportunity, especially since there were and are many gray areas around SARS-CoV-2. Therefore, we have expanded our thesis objectives to include questions relating to SARS-CoV-2.

Indeed, before declaring the end of the 10th EVD outbreak in the DRC on June 25, 2020, the country reported its first case of COVID-19 on March 10, 2020 in Kinshasa, the capital of the DRC. In fact, COVID-19 is a disease caused by SARS-CoV-2, which was first identified in early December in Wuhan city, Hubei province of China and declared a pandemic by the WHO on March 11, 2020. Coronaviruses are enveloped RNA viruses that are prevalent in humans, other mammals, and birds and cause respiratory, intestinal, liver, and neurological diseases. Seven species of coronavirus are known to cause disease in humans. Four viruses (229E, OC43, NL63, and HKU1) are prevalent and commonly cause cold symptoms in immunocompetent people, however, the other two strains, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), are zoonotic and sometimes associated with fatal diseases. For example, SARS-CoV caused severe acute respiratory syndrome in Guangdong Province, China, in 2002 and 2003 while MERS-CoV was the causative agent of severe respiratory disease in the Middle East and South Korea in 2012-2013. The seventh strain, the newly discovered coronavirus named first novel CoV (2019-nCoV) is a β -coronavirus and it is responsible of the current global pandemic. The virus was named SARS-CoV-2 and the disease COVID 19 by WHO on February 11, 2020. In DRC, the COVID-19 pandemic response is under the responsibility of the Technical Secretariat of the Response against COVID-19.

Emerging and re-emerging pathogens are global public health issues. Therefore, scientific studies on EIDs are important for studying their conditions, understanding prevention tools and developing treatments. For example, during the tenth EVD epidemic, new response strategies were put in place to quickly diagnose the disease, limit its spread and improve the care of infected people. Among these strategies, the first step was diagnosis using the geneXpert platform, a highly sensitive and specific PCR test that provides results within 2 hours of collection. It was deployed via mobile laboratories at 10 different sites with a total of 238,648 samples analyzed (Figure 16). A high-throughput sequencing platform has been set up at the National Institute for Biomedical Research (INRB) in Kinshasa to rapidly characterize epidemics and monitor transmission chains. Secondly, ring vaccination was conducted around confirmed cases (contacts of confirmed cases and contacts of contacts) and frontline health workers. In total, up to 300,000 doses of rVSV-ZEBOV-GP vaccine have been administered. Third, the use of experimental molecules, both in compassionate

use protocols (MEURI) and in randomized clinical trials. From November 2018 to August 9, 2018, 4 centers participated in randomized clinical trials (Mangina, Beni, Butembo and Katwa) with a total of 681 participants. Four molecules were used, including 3 monoclonal antibodies (ZMapp-52, Inmazed-128 and Ansuvimab-128 (Ebanga) and 1 antiviral agent (Remdesivir-50). Two molecules (Inmazed and Ansuvimab) showed good efficacy and were used for the treatment of new patients with EVD.

It is true that these new strategies have improved the response to this epidemic, but they have also raised several questions that deserve further investigations. On the one hand, because the case definition used in EVD is very broad and thus less specific, mobile laboratories in the field have performed a large number of molecular tests (GeneXpert) on patients with clinical symptoms who have turned out to be negative. However, it is possible that some of these suspected cases were Ebola patients with less severe symptoms who arrived at health centers after the acute phase, in part because testing centers were inaccessible due to the insecurity situation. In a pilot study we conducted at the INRB with samples from 200 patients with clinical symptoms but negative with genXpert, three samples were tested positive for Ebola antibodies (IgG and IgM), indicating the presence of unknown transmission chains. Additionally, suspected Ebola-negative patients may have other infectious etiologies. On the other hand, it is important to evaluate the impact of the administered treatment on the carrier status of the virus (in sperm and breast milk) and on antibody dynamics compared to untreated patient cohorts previously reported in Guinea.

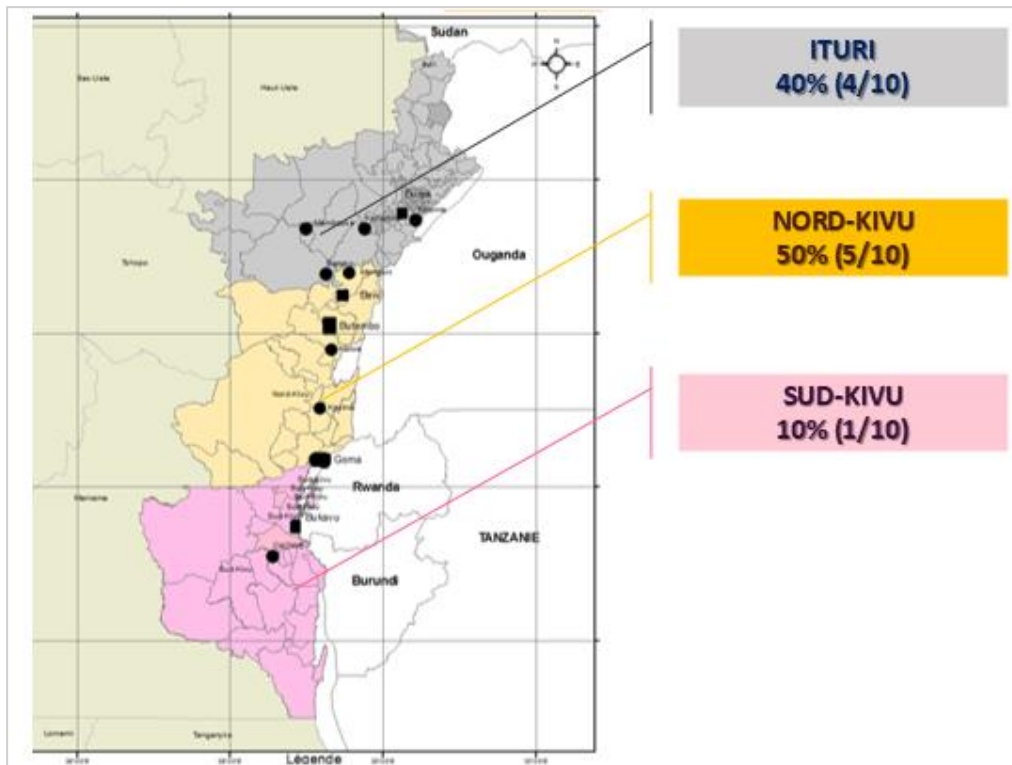


Figure 16. Sites of deployment of mobile Ebolavirus laboratories in Eastern DR Congo.

The evolution of the COVID-19 disease in the DRC has raised many questions that require in-depth investigations. For example, a mathematical estimate study predicted more than 70,000,000 cases and more than 3,000,000 deaths in the first year the virus circulated in Africa. In particular, these assessments considered the lack of infrastructure to better manage severe cases of COVID-19 in Africa. However, six months after the declaration of the COVID-19 pandemic in DRC, and after the first wave, only 11,078 confirmed COVID-19 cases were reported, with 303 deaths in the country with around 90,000,000 inhabitants. These reported number of cases and deaths were much lower than those predicted, suggesting a heterogeneity in the global COVID-19 epidemic. Thus, given the recommendations of WHO in the context of Africa, population-based sero-surveillance should be conducted to complete the reported cases of COVID-19 via RT-PCR and estimate the true extent of the disease and take the necessary public health measures. Several serological studies have been conducted in Africa and have shown wide variability in SARS-CoV-2 seroprevalence in different countries, but most have been conducted in specific populations such as blood donors, healthcare workers or other high-risk

populations. In addition to epidemiological factors, the differences in reported seroprevalence can be explained by unique immunoglobulin G (IgG) antibodies and different types of serological tests used to screen target populations.

The thesis is subdivided into two parts:

The first part: Description and evaluation of human humoral immune response against EBOV. This part will elucidate the following research questions:

1. There were 90% of suspected EVD patients but discharged as negative after two consecutive negative RT-PCR. The research question is what is the prevalence of EVD cases among these patients?
2. EVD patients were treated using specific newly developed molecules. What is the impact of these molecules on the dynamic of the humoral immune response in patients who recovered from EVD?

1.2. Part 1: general objective

The first part aimed to characterize the human humoral immune response against the Ebola virus in order to understand the place occupied by pauci-symptomatic EVD infection during an epidemic, and to evaluate the impact of new treatments used during the 10th EVD outbreak in DRC.

1.2.1. Part 1: specific objectives

1. Determine the frequency of anti-Ebola virus IgG among suspected EVD patients but discharged as negative by RT-PCR;
2. Determine the immunological profile of EBOV survivors and evaluation the impact of the treatments received on the immune response compared with those of the epidemic in Guinea;

The second part: Description and evaluation of human humoral immune response against SARS-CoV-2. This part will elucidate the following research questions:

1. One of the reasons of the variability in reporting SARS-CoV-2 seroprevalence in Africa is the different types of serological tests used. Which serological test should be suggested for a better estimation of the seroprevalence?
2. The reported RT-PCR cases of SARS-CoV-2 infections were much lower than those expected. What is the extent of the COVID 19 in Kinshasa, the capital city of the DRC after the first wave of the pandemic in the country?

1.3. Part 2: general objective

In this second part, we aimed to understand the true extent of SARS-CoV-2 in Kinshasa the capital of the DRC, and to provide these results and information to the public health authorities for policy change and adaptation of outbreak response to real needs.

1.3.1. Part 2: specific objectives

1. Develop and evaluate the serological test for the detection of antibodies against SARS-CoV-2 in humans;
2. Determine the prevalence of anti-SARS-CoV-2 IgG antibodies in the general population in Kinshasa.

Chapter 2. Material and Methods

2.1. Ebola studies

2.1.1. Ebola non-case study database

We conducted a retrospective study on left-over samples of clinically Ebola suspected patients diagnosed as negative during the EVD epidemic in the DRC. We used data and samples that were collected during the 10th Ebola outbreak reported in the Eastern provinces of the DRC. The samples were stored in the biobank of the INRB laboratory in Kinshasa/DRC.

2.1.2. Study of Ebola antibody kinetics in survivors

We conducted a retrospective cohort study using a large cohort of Ebola survivors called “Vainqueurs d’Ebola” conducted by the Programme National de Suivi des Personnes Guéries (PNSPG) in collaboration with the INRB, INSERM Paris, Institut Bouisson Bertrand (IBB), and Institut de Recherche pour le Développement (IRD). The vainqueurs d’Ebola project was established for recruitment of the EVD cured patients for clinical and psychological care. The clinical and sociodemographic data of the patients were collected during the follow-up and the patients were asked to provide the blood samples after giving their consent. The samples were stored at -80°C in the biobank of the INRB. The full protocol of the project can be found in the annex 1.

2.2. Studies on SARS-CoV-2

2.2.1. Development of multiplex serological test

The human samples and the corresponding data were provided by consenting COVID-19 patients who were hospitalized in Montpellier University Hospitals. We also constitute the negative panel using the samples collected from leftover diagnostic samples of the virology laboratory of the University Hospital in Montpellier, France.

2.2.2. SARS-CoV-2 seroprevalence studies

We used data from two studies that have been conducted in Kinshasa the capital city of the DRC. The first study was a prospective cohort study conducted in health care workers and their related households. Participants were asked to provide samples in four points during 6 months (the full protocol can be found in annex 2). The second study called ARIACOV was cross sectional study conducted in general population in Kinshasa after the first wave of COVID-19 (the full protocol is listed in annex 3).

2.3. Methods

2.3.1. Antibody detection

We used xMAP® technology as the primary antibody detection tool for Ebola and SARS-CoV-2. The xMAP technology invented in the 1990s by Luminex scientists combines fluidics, optics and digital signal processing with proprietary microsphere beads and enables a high degree of multiplexing in a single sample volume. The magnetic polystyrene beads sized 5.6 µm of diameter contain two fluorochromes and have on their surface the carboxylate groups that act as solid phase and enable covalent link to the amino groups found in purified antigens, capture antibodies or nucleic probes characteristic of particular alleles. For EBOV we covalently coupled beads to four commercially available recombinant proteins (1 µg/1.25 × 10⁶ beads): two *glycoproteins*, GP-EBOV-k (*Kissidougou* strain 2014) and GP-EBOV-m (*Mayinga* strain 1976); one *nucleoprotein*, NP-EBOV (*Kissidougou* strain 2014); and one 40-kDa *viral protein* (VP40-EBOV, *Kissidougou* strain 2014) and for SARS-CoV-2, we used commercially available recombinant proteins Spike (S1) and Nucleocapsid (NP). Following the principal of indirect ELISA, we used plasma samples in a dilution of 1/1000 in dilution buffer for EBOV and 1/200 for SARS-CoV-2 in a mixture with appropriate coupled beads. Once the assay is complete, the solid phase characteristics allow each bead to be analyzed discretely and the magnetic properties into xMAP microspheres maintain desirable solution-phase properties while we process with washing steps of the assay. Using cytometric principal, a suspension of beads undergoes aspiration, then travels through a liquid vein and at the exit of the liquid vein, the beads are excited by a set of two lasers or led light, each having a specific role. The red laser (633 nm), which excites the fluorochromes incorporated into the polystyrene

beads emitting in the red and infrared, corresponding to the color code of each bead, and allowing the precise identification of each. The green laser, which excites the fluorochrome bound to a reporter molecule (anti-immunoglobulin or amplification product), allows to detect the interaction antigen-antibody or probe-amplicon on the surface of the bead, after adding a conjugate labeled with a fluorochrome radiating green. In general, it is phycoerythrin which, excited at 532 nm, is re-emitted at 575 nm. The fluorescence emitted therefore testifies to the reaction on the surface and determines the positivity of the reaction according to a specific fluorescence threshold for each bead. The 96 wells plates are read using Luminex device (Magpix or Bioplex) using xPONENT software. Results are recorded as Median Fluorescence Intensity (MFI) of each analyte ([Angeloni S *et al.*, 2022](#); [Moalic *et al.*, 2004](#)).

In addition to the Luminex methods, we have also used other serological diagnostic methods for the detection of anti-SARS-CoV-2 antibodies. We used the conventional commercial Euroimmun IgG ELISA (Euroimmun, Lübeck, Germany), and Abbott SARS-CoV-2 IgG for Alinity Enzyme immunoassay (EIA). We also used a point of care rapid test Quickzen SARS-CoV-2 IgG and IgM (Zentech, Belgium). Furthermore, viral sequencing was performed on the samples of EVD relapse case using Illumina iSeq 100 for 2×150 cycles or Illumina MiSeq for 2×150 cycles at the INRB on the samples of the EVD relapse cases. The anti-human Ebola GP IgG ELISA was performed on the EVD relapse case samples using the respective Alpha Diagnostic International kits.

2.3.2. Interpretation of the xMAP results

Luminex results are continuous values and are exported as Comma Separated Values (CSV) files. The results are analyzed quantitatively and qualitatively. Therefore, a qualitative interpretation of the results requires the establishment of threshold values. For EBOV, we used the cut-off that have been calculated previously on a large panel of survivors and their contacts in Guinea. The cut-off values were 600 for NP, 400 for GP, and 650 for VP-40 proteins ([A. Ayouba *et al.*, 2017](#); [Diallo *et al.*, 2019](#)). However, we calculated and established the cut-off values for SARS-CoV-2 proteins using Mean + 3SD formula calculated on the panel of confirmed SARS-CoV-2 negative samples. We compared the obtained cut-off values to that we calculated using the receiver operating characteristics

(ROC) curve analysis. Finally, and based on the profile of EVD survivors from Guinea, we set as positivity criterion a simultaneous reactivity of a sample to at least two antigens for EBOV. For SARS-CoV-2, we also have seen a variable reactivity to a single SP antigen mostly in the samples with high level of background. Therefore, we also considered as positive each sample that reacts to SP and NP simultaneously.

Thesis part 1: Characterization of anti-Ebola virus humoral immune response

Chapter 1. Prevalence of Ebola infection among clinically suspected patients

1.1. Added Value of an Anti-Ebola Serology for the Management of Clinically Suspected Ebola Virus Disease Patients Discharged as Negative in an Epidemic Context

Antoine Nkuba-Ndaye,^{1,2,3} Daniel Mukadi-Bamuleka,^{2,3} Junior Bulabula-Penge,^{2,3} Guillaume Thaurignac,¹ François Edidi-Atani,^{2,3} Fabrice Mambu-Mbika,^{2,3} Bernice Danga-Yema,³ Meris Matondo-Kuamfumu,^{2,3} Eddy Kinganda-Lusamaki,^{1,2,3} Nella Bisento,² Raphaël Lumembe-Numbi,^{2,3} Gabriel Kabamba-Lungenyi,^{2,3} Divine Kitsa-Mutsumbirwa,⁴ Nelson Kambale-Sivihwa,⁵ François Boillot,⁶ Eric Delaporte,¹ Placide Mbala-Kingebeni,^{2,3} Ahidjo Ayouba,¹ Martine Peeters,¹ and Steve Ahuka-Mundeke^{2,3}

¹Recherches Translationnelles sur le Virus de l'Immunodéficience Humaine et Maladies Infectieuses, Université de Montpellier/Institut National de la Santé et de la Recherche Médicale U1175, Institut de Recherche pour le Développement, Montpellier, France, ²Département de Virologie, Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, ³Département de Biologie Médicale, Université de Kinshasa, Kinshasa, Democratic Republic of the Congo, ⁴Laboratoire Mobile pour Maladie à Virus Ebola, Institut National de Recherche Biomédicale, Butembo, Nord-Kivu, Democratic Republic of the Congo, ⁵Laboratoire Mobile pour Maladie à Virus Ebola, Institut National de Recherche Biomédicale, Beni, Nord-Kivu, Democratic Republic of the Congo, ⁶Alter-Santé Internationale & Développement, Montpellier, France

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Correspondence: Antoine Nkuba-Ndaye, MD, MPH, Institut de Recherche pour le Développement, Université de Montpellier, 911 Avenue Agropolis, 34394 Montpellier Cedex 5, France (antoinnkuba@gmail.com).

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Background. Survivors from Ebola virus disease (EVD) may be at the origin of EVD resurgence.

Methods. Left-over of blood samples selected randomly from suspected EVD patients but discharged as negative were analyzed using a highly sensitive and specific Luminex® for detection of anti-IgG antibodies.

Results. Simultaneous reactivity to at least 2 *Ebola virus* or *Zaire ebolavirus* (EBOV) antigens was detected in 11 of 488 (2.3%; 95% confidence interval [CI], 1.1–4.0) suspected EVD patients who were discharged as negative after 2 consecutive negative tests during the 10th *Ebola* outbreak in the Democratic Republic of the Congo. After extrapolating the total number of individuals discharged as negative during the entire outbreak, we estimated a total of 1314 additional missed *Ebola* cases.

Conclusions. These findings emphasize the usefulness of an EBOV serology analysis and the importance of extending epidemic surveillance to clinically suspected cases who were discharged as negative.

Keywords. DRC; *Ebola*; Ituri; North-Kivu; serology.

1.1.1. Introduction

Ebolaviruses are among the most virulent viruses and has a high mortality rate. Since the first recognition of *Zaire ebolavirus* (EBOV) in 1976 in the Democratic Republic of the Congo (DRC), the country has registered 13 outbreaks (CDC, 2021b). The 10th Ebola virus disease (EVD) outbreak in Eastern DRC, declared on first August 2018, was the longest (2 years) and the most devastating ever recorded in the country with a total of 3470 cases with 2287 deaths (WHO, 2020a). Although it is generally assumed that each EVD outbreak results from an independent zoonotic transmission of the virus from wildlife to humans (Pigott *et al.*, 2014), recently 2 out-breaks in DRC have resurged from individuals who recovered from EVD (Mbala-Kingebeni *et al.*, 2021). Moreover, genomic analysis of the 2021 EVD outbreak in Guinea showed that resurgence from humans can even occur more than 5 years after the end of the epidemic (Keita *et al.*, 2021). During the large EVD outbreak in West Africa (2013–2016), several independent studies have shown that some EBOV infections remain asymptomatic or paucisymptomatic and therefore go undiagnosed (Diallo *et al.*, 2019; Glynn *et al.*, 2017). As part of the outbreak response in Eastern DRC, a recombinant vesicular stomatitis virus-*Zaire ebolavirus* vaccine (rVSV-ZEBOV-GP, Mayinga strain 1976) has been widely used in a ring vaccination strategy to prevent transmission (WHO, 2020a) that may increase the possibility of developing mild Ebola virus infection. In addition, insecurity in this affected area may have delayed access to reverse-transcription polymerase chain reaction (RT-PCR) testing, and some patients could have been referred to the Ebola treatment center after the viremic period. More importantly, viral relapse or resurgence due to the persistence of the virus in body fluids, such as semen and breast milk, or in immune-privileged sites can also occur in undiagnosed EVD patients (Keita *et al.*, 2021). To estimate the number of possible missed EVD patients during the 10th EBOV outbreak in Eastern DRC, we studied the presence of antibodies for EBOV in clinically suspected EVD cases with a negative RT-PCR test result.

1.1.2. Patients and methods

Study Design and Sample Collection

We performed a retrospective study on leftover blood samples of clinically suspected EVD patients who tested negative in 2 consecutive EBOV RT-PCR tests. Samples were collected

in the 3 provinces affected by the 10th Ebola outbreak in the DRC, that is, North-Kivu, South-Kivu, and Ituri. Samples were centralized and stored frozen in the Biobank of the Institut National de Recherche Biomédicale. We used the national EBOV database containing clinical, demographic, and laboratory results of available blood samples (132 051) of 4 health zones where almost all confirmed cases >90% (3315 of 3461) occurred (Figure 17): Beni, Butembo, Katwa, and Mabalako. With the Random function of Microsoft Office Excel 2016, we selected 600 samples corresponding to the second negative EBOV RT-PCR samples. For a control group, we used archived samples collected in 2011 as part of human immunodeficiency virus monitoring in the same geographic area before Ebola vaccination was used or an EVD outbreak was reported (Boillot *et al.*, 2016). We considered clinical variables related to acute phase symptoms, epidemiological variables (contact to index case), and sociodemographic variables (age, sex, and diagnostic site).

Laboratory Analysis

Serological analysis was performed using the Luminex technology (Luminex Corp., Austin, TX) and following the protocol previously published (A. Ayouba *et al.*, 2017; Diallo *et al.*, 2019). In brief, 4 commercially available recombinant EBOV proteins were coupled to magnetic beads ; 2 glycoproteins, GP-EBOV-k (Kissidougou strain 2014) and GP-EBOV-m (Mayinga strain 1976) ; 1 nucleoprotein, NP-EBOV (Kissidougou strain 2014) ; and 1 40-kDa viral protein (VP40-EBOV, Kissidougou strain 2014). Antigens coupled to beads were mixed with the patient sample (1/1000 sample to dilution buffer), and the reaction signal for anti-EBOV immunoglobulin G (anti-IgG) was read and recorded on Bio-Plex 200 equipment (Bio-Rad, Marnes-la-Coquette, France). Results were expressed as median fluorescence intensity. Samples with positive signal to at least 2 antigens simultaneously were considered as positive EVD cases (A. Ayouba *et al.*, 2017).

Statistical Analysis

We expressed categorical data in frequencies, whereas continuous data were expressed in median and quantiles. Chi-square Fisher's exact tests were used. We considered $P < .05$ as the significant threshold.

Ethical Considerations

The sample collection process in the field was exempted by the review from the ethical committee, because it was part of the EBOV outbreak response. Permission to analyze and use data was obtained by the Ethical Committee of the Ecole de Santé Publique de l’Université de Kinshasa (ESP-UNIKIN, Number ESP/CE/172/2021).

1.1.3. Results

Sociodemographic Characteristics of Clinically Suspected Ebola Virus Disease Patients

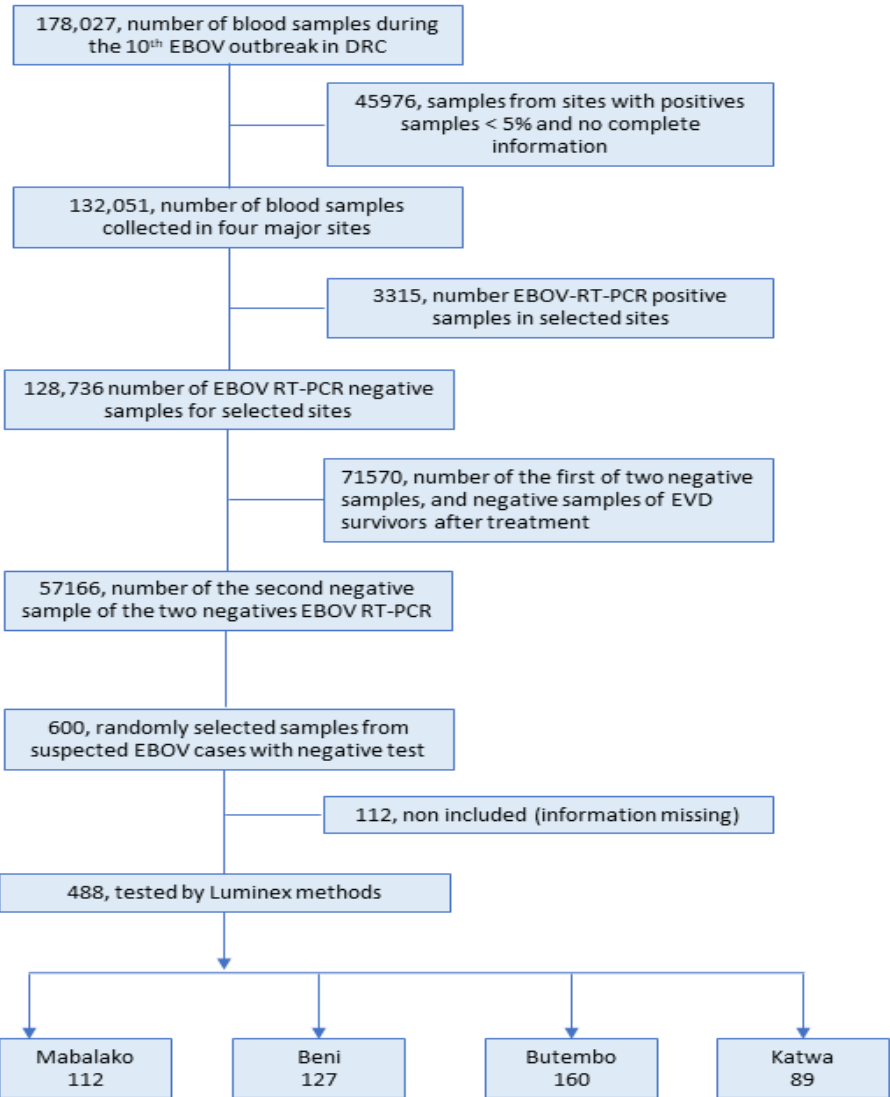


Figure 17. Flowchart of clinical Ebola virus disease (EVD) suspected patients and their stored samples selection for 4 health zones: Beni, Mabalako, Butembo, and Katwa.

DRC, Democratic Republic of the Congo; EBOV, *Zaire ebolavirus*; RT-PCR, reverse-transcription polymerase chain reaction.

Among the 600 randomly selected samples from negative EBOV RT-PCR samples, 488 with sufficient clinical information were tested for EBOV antibodies. Among these, 127 samples were from Beni, 160 from Butembo, 112 from Mabalako, and 89 from Katwa (Figure 17); 244 (50.3%) and 241 (49.7%) were from male and female patients, respectively. There was no age and sex information for 3 individuals. The median age was 22 years (interquartile ranges, 12-32), and 86.6% (420 of 485) were aged below 40 years (Table 5).

Serological Characteristics of Clinically Suspected Ebola Virus Disease Patients

Of 488 samples analyzed, 7 (1.4%) were reactive with NP antigen, 54 (11.1%) with GP, and 39 (8%) with VP40 versus 2 (0.7%), 15 (5.4%), and 5 (1.8%) to NP, GP, and VP40 EBOV antigens, respectively, in the control group. Differences were significant for GP and VP40 EBOV antigens ($P < .01$) (Table 4). By applying the previously defined positivity criteria for EBOV infection with this assay, that is, simultaneous reactivity to at least 2 antigens, 11 (2.3%; 95% CI, 1.1–4.0) (Table 4) clinically suspected EVD patients were positive versus 1 sample in the control group (0.4%) ($P = .064$). We observed 2 (0.44%) samples positive to NP and GP, 5 (1.0%) samples positive to GP and VP40, 1 (0.2%) sample positive to NP and VP40, and 3 (0.6%) samples that had IgG antibodies to NP, GP, and VP40 EBOV antigens simultaneously. During the outbreak, mobile laboratories in Beni, Butembo, Mabalako, and Katwa received and analyzed 132 051 blood samples from patients with clinical suspicion of EVD, and only 3315 (2.5%) were confirmed positive by PCR (unpublished observations). Because the rate of patients positive for at least 2 antigens was 2.3% in our study, we extrapolated that 1314 additional patients could have EBOV antibodies among the 57 166 who were tested twice and discharged as negative and thus could be undiagnosed EVD patients (Table 6).

Table 4. Proportion of Anti-IgG EBOV Antibodies for NP, GP, and Matrix Protein VP-40

Variables (EBOV Antigens)	Ebola Suspected Cases (n = 488)		Negative Control (n = 280)		P Value
	n Positive	% (95% CI)	n Positive	% (95% CI)	
NP	7	1.4 (0.6–2.9)	2	0.7 (0.0–2.6)	.498
GP	54	11.1 (8.4–14.2)	15	5.4 (3.0–8.7)	.007 ^a
VP-40	39	8.0 (5.7–10.8)	5	1.8 (0.6–4.1)	.001 ^a
At least 2 antigens	11	2.3 (1.1–4.0)	1	0.4 (0.0–2.0)	.064
NP + GP	2	0.4 (0.0–1.4)	0	0.0 (0.0–1.3)	.536
GP + VP-40	5	1.0 (0.3–2.4)	1	0.4 (0.0–2.0)	.424
NP + VP-40	1	0.2 (0.0–1.1)	0	0.0 (0.0–1.3)	.999
NP + GP + VP-40	3	0.6 (0.0–1.8)	0	0.0 (0.0–1.3)	.557

Abbreviations: CI, confidence interval; EBOV, *Zaire ebolavirus*; GP, glycoprotein; NP, nucleocapsid.

^aSignificant difference at 1%.

Clinical Characteristics of the Clinically Suspected Ebola Virus Disease Patients

The case definition used for active surveillance was broad and unspecific, and symptoms reported by clinically suspected EVD patients were not specific to only EBOV infection. We analyzed all reported symptoms when admitted for EBOV RT-PCR test and assessed whether some were associated with antibody positivity, that is, presence of antibodies to at least 1 EBOV antigen. Symptoms such as asthenia 312 (63.9%), headache 299 (61.3%), anorexia 278 (57%), fever 262 (53.7%), abdominal pain 245 (50.2%), and arthralgia 166 (34%) were the most prevalent symptoms expressed. Antibody positivity was not associated with particular clinical symptoms ($P > .05$) (Table 7).

Information on epidemiological link with confirmed EVD patients was available for 324 patients. Reactivity with 2 EBOV antigens was higher in patients reporting a link (2 of 46; 4.3%) versus those who did not (6 of 278; 2.2%), but the difference was not significant ($P > .05$) (Table 7). Among patients positive for at least 2 EBOV antigens, 6 (54.5%) reported symptom onset of less than 6 days, and 5 (45.5%) reported symptom onset greater than 6 days (Table 8).

1.1.4. Discussion

There is increasing evidence that EVD can re-emerge in patients who recovered from the disease, as illustrated in 2021 by at least 2 relapses from the 10th EBOV outbreak that ended in August 2020 in the DRC, but also from reports during the 11th outbreak in the Equateur

province in 2020 in the DRC, and from the outbreak in 2021 in Guinea, West Africa (Keita *et al.*, 2021; Mbala-Kingebeni *et al.*, 2021; WHO, 2020a). With the magnitude of 2 recent outbreaks in Eastern DRC and West Africa, the number of patients who recovered from the disease increased and thus also the risk for re-emergence from humans. Therefore, it is important to estimate the proportion of possible missed EVD patients.

The 10th EBOV outbreak in the DRC occurred in a region experiencing civil war for more than a decade, which hindered the EVD response team to list and perform follow-up of all clinically suspected EVD patients during certain periods (Wells *et al.*, 2019). Therefore, we cannot exclude that national security challenges also delayed access to diagnostic and clinical care. In the present study, we determined the presence of anti-EBOV antibodies in patients who attended the clinics with clinical symptoms of suspected EVD but tested negative after 2 consecutive RT-PCR tests during this outbreak.

We found that 2.3% (11 of 488) of samples were positive for anti-IgG antibodies to at least 2 EBOV antigens, which suggests previous EBOV infection. After extrapolating data for 57 166 individuals who were discharged as negative during the outbreak, we estimate that 1314 additional patients may have recovered from EBOV infection. This number is likely underestimated because asymptomatic household contacts of EVD cases were not tested for EBOV antibodies, and this was also not done for the additional cases that we uncovered in this report. During the West African outbreak, viral infection was reported in 2.6% (10 of 388) of household contacts without clinical symptoms related to EVD in Sierra Leone (Glynn *et al.*, 2017; Kuhn *et al.*, 2017). Among highly exposed contacts, even 7.5% (20 of 267) with positive serology for at least 2 antigens were reported in Sierra Leone (Halfmann *et al.*, 2019). Another study in Guinea reported that 4% (57 of 1390) of asymptomatic and paucisymptomatic contacts of EBOV patients had antibodies (Diallo *et al.*, 2019). It is interesting to note that a study conducted on 24 close contacts of symptomatic patients in Gabon showed that 11 (45.8%) patients developed both IgM and IgG response to EBOV antigens, indicating viral infection confirmed by detection of genomic ribonucleic acid in peripheral blood mononuclear cells (Leroy *et al.*, 2000), but there are no follow-up data for these patients.

An additional factor of underestimation is that we did not test for IgM antibodies, although IgG antibodies start to develop rapidly after symptom onset and are generally present after viral clearance from blood (Ploquin *et al.*, 2018). In analogous observations with the same antibody assay in EVD survivors in Guinea showing simultaneous presence of IgG antibodies to multiple antigens after EBOV infection in 99% of patients who recovered (Diallo *et al.*, 2021), we used the same criteria in our study. It is possible that this could be too strict shortly after symptom onset especially in paucisymptomatic patients for whom almost no data on antibody kinetics are available (Ploquin *et al.*, 2018). Finally, we cannot exclude the possibility that those IgG-positive patients are from an unnoticed outbreak in the past. Therefore, we compared our samples with samples from 2011 in the same area, and 1 (0.4%) sample had antibodies to GP and VP40 simultaneously. Moreover, the proportion of reactivity to a single antigen was also significantly lower. The higher proportion of individuals with antibodies to GP antigens could be partially due to the vaccination strategy with the rVSV-ZEBOV-GP during the outbreak, which induces antibodies to GP proteins only, but information on vaccination was not recorded for the samples analyzed in our study and thus could not be further analyzed.

1.1.5. Conclusions

Despite the broad and unspecific case definition used for active surveillance to not miss any EBOV infection during the outbreak, more than 1000 additional EBOV cases could have gone undetected, that is, approximately one third of the official number of RT-PCR-confirmed cases. More importantly, the index case in the EVD outbreak in February 2021 in Guinea was not registered as an EVD survivor, whereas the genomic analysis of the virus showed that the virus strain was related to the previous outbreak (Keita *et al.*, 2021). This is the first study on anti-EBOV antibodies among clinically suspected EVD patients who were discharged as negative after RT-PCR tests, and it suggests that the proportion of paucisymptomatic EBOV infection can be higher than suspected from previous outbreaks. These findings emphasize the importance of extending epidemic surveillance during and after outbreaks to related EVD household contacts and to suspected cases discharged as negative with an additional accurate and specific serological assay, which ideally can also discriminate vaccine-related antibodies given the large use of vaccines in EVD outbreak response.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

1.1.6. Supplementary tables

Table 5. General characteristics of suspected EVD patients

Variables	EVD suspect cases			Negative control group		
	Female	Male	Total	Female	Male	Total
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Age, y						
0 – 5	35(54.7)	29(45.3)	64(13.3)	0	0	0
6 – 17	53(47.3)	59(52.7)	112(23.1)	0	0	0
18 – 39	122(50.0)	122(50.0)	244(50.3)	116(73.0)	43(36.0)	159(57.4)
≥40	31(47.7)	34(52.3)	65(13.4)	75(63.6)	43(36.4)	118(42.6)
Age						
(median, 1 st , 21 st quantiles)	21[13-34]	22.25[13.2-45]	22[12-32]	36[30-43]	39[35-46,8]	22[12-32]
Total[†]						
	241(49.7)	244(50.3)	485	191(69.0)	86(31.0)	277

[†]Age and sex are summarized for 485 clinical suspect EVD patients. There was no information for three patients.

Table 6. Estimation of missed EBOV cases among suspected EVD cases discharged as negative after two PCR tests

	Beni		Butembo		Katwa		Mabalako		Total	
	n	%	n	%	n	%	n	%	n	% (CI)
Number of samples tested (10 th EVD outbreak)	44459	33,7	46605	35,3	21698	16,4	19289	14,6	132051	
Positive samples by RT-PCR	924	2,1	1129	2,4	542	2,5	720	3,7	3315	2,5 (2,4-2,6)
All negative samples by RT-PCR	43535	97,9	45476	97,6	21156	97,5	18569	96,3	128736	97,5
Only second negative sample by RT-PCR using for discharge	26256	59,1	14699	31,5	11116	51,2	5095	26,4	57166	43,3
Selected samples	127	0,5	160	1,1	89	0,8	112	2,2	488	0,9
Positive samples for at least 2 anti-IgG EBOV	3	2,4	5	3,1	0	0,0	3	2,7	11	2,3 (1,1-4,0)
Estimated number of missed EVD infections									1314	

Table 7. Clinical characteristics of Ebola suspected cases and antibody positivity

Symptoms	Number of tests 488	Anti-EBOV NP IgG		<i>p</i> -value	Anti-EBOV GP IgG		<i>p</i> -value	Anti-EBOV VP40 IgG		<i>p</i> -value	At least two antigens		<i>p</i> -value
		Negative n(%)	Positive n(%)		Negative n(%)	Positive n(%)		Negative n(%)	Positive n(%)		Negative n(%)	Positive n(%)	
Fever													
No		222(46,2)	4(57,1)	0,831	207(47,7)	19(35,2)	0,082	211(47,0)	15(38,5)	0,305	222(46,5)	4(36,4)	0,556
Yes		259(53,8)	3(42,9)		227(52,3)	35(64,8)		238(53,0)	24(61,5)		255(53,5)	7(63,6)	
Nausea													
No		258(53,6)	4(57,1)	0,71	236(54,4)	26(48,1)	0,386	244(54,3)	18(46,2)	0,325	254(53,2)	8(72,7)	0,235
Yes		223(46,4)	3(42,9)		198(45,6)	28(51,9)		205(45,7)	21(53,8)		223(46,8)	3(27,3)	
Diarrhoea													
No		360(74,8)	6(85,7)	0,868	328(75,6)	38(70,4)	0,404	341(75,9)	25(64,1)	0,101	358(75,1)	8(72,7)	0,714
Yes		121(25,2)	1(14,3)		106(24,4)	16(29,6)		108(24,1)	14(35,9)		119(24,9)	3(27,3)	
Asthenia													
No		174(36,2)	2(28,6)	0,507	153(35,3)	23(42,6)	0,289	165(36,2)	11(28,2)	0,286	172(36,1)	4(36,4)	0,605
Yes		307(63,8)	5(71,4)		281(64,7)	31(57,4)		284(63,3)	28(71,8)		305(63,9)	7(63,6)	
Anorexia													
No		209(43,5)	1(14,3)	0,12	190(43,8)	20(37,0)	0,345	196(43,7)	14(35,9)	0,348	206(43,2)	4(36,4)	0,764
Yes		272(56,5)	6(85,7)		244(56,2)	34(63,0)		253(56,3)	25(64,1)		271(56,8)	7(63,6)	
Abdominal pain													
No		238(49,5)	5(71,4)	0,94	219(50,5)	24(44,4)	0,404	230(51,2)	13(33,3)	0,032*	237(49,7)	6(54,5)	0,749
Yes		243(50,5)	2(28,6)		215(49,5)	30(55,6)		219(48,8)	26(66,7)		240(50,3)	5(45,5)	
Chest pain													
No		437(90,9)	6(85,7)	0,494	395(91,0)	48(88,9)	0,61	413(92,0)	30(76,9)	0,998	434(91,0)	9(81,8)	0,269
Yes		44(9,1)	1(14,3)		39(9,0)	6(11,1)		36(8,0)	9(23,1)		43(9,0)	2(18,2)	
Myalgia													

No	371(77,1)	4(57,1)	0,205	337(77,6)	38(70,4)	0,231	349(77,7)	26(66,7)	0,116	369(77,4)	6(54,5)	0,076
Yes	110(22,9)	3(42,9)		97(22,4)	16(29,6)		100(22,3)	13(33,3)		108(22,1)	5(45,5)	
Arthralgia												
No	318(66,1)	4(57,1)	0,445	287(66,1)	35(64,8)	0,847	304(67,7)	18(46,2)	0,006*	316(66,2)	6(54,5)	0,417
Yes	163(33,9)	3(42,9)		147(33,9)	19(35,2)		145(32,3)	21(53,8)		161(33,8)	5(45,5)	
Headache												
No	187(38,9)	2(28,6)	0,447	171(39,4)	18(33,3)	0,388	179(39,9)	10(25,6)	0,08	186(39,0)	3(27,3)	0,541
Yes	294(61,1)	5(71,4)		263(60,6)	36(66,7)		270(60,1)	29(74,4)		291(61,0)	8(72,7)	
Cough												
No	350(72,8)	7(100)	0,999	321(74,0)	36(66,7)	0,253	331(72,7)	26(66,7)	0,34	348(73,0)	9(81,8)	0,735
Yes	131(27,2)	0(0)		113(26,0)	18(33,3)		118(26,3)	13(33,3)		129(27,0)	2(18,2)	
Breathlessness												
No	449(93,3)	6(85,7)	0,389	405(93,3)	50(92,6)	0,508	421(93,8)	34(87,2)	0,116	446(93,5)	9(81,8)	0,166
Yes	32(6,7)	1(14,3)		29(6,7)	4(7,4)		28(6,2)	5(12,8)		31(6,5)	2(18,2)	
Swallowing problem												
No	455(94,6)	7(100)	0,999	408(94,0)	54(100)	0,098	426(94,9)	36(92,3)	0,453	451(94,5)	11(100)	0,999
Yes	26(5,4)	0(0)		26(6,0)	0(0)		23(5,1)	3(7,7)		26(5,5)	0(0)	
Sore throat												
No	465(96,7)	7(100)	0,999	418(96,3)	54(100)	0,237	434(96,7)	38(97,4)	0,999	461(96,6)	11(100)	0,999
Yes	16(3,3)	0(0)		16(3,7)	0(0)		15(3,3)	1(2,6)		16(3,4)	0(0)	
Jaundice												
No	474(98,5)	7(100)	0,999	427(98,4)	54(100)	0,999	444(98,9)	37(94,9)	0,101	470(98,5)	11(100)	0,999
Yes	7(1,5)	0(0)		7(1,6)	0(0)		5(1,1)	2(5,1)		7(1,5)	0(0)	
Conjunctivitis												
No	473(98,3)	6(85,7)	0,122	427(98,4)	52(96,3)	0,261	442(98,4)	37(94,9)	0,156	469(98,3)	10(90,9)	0,186
Yes	8(1,7)	1(14,3)		7(1,6)	2(3,7)		7(1,6)	2(5,1)		8(1,7)	1(9,1)	
Skin rash												

No	468(97,3)	7(100)	0,999	424(97,7)	51(94,4)	0,164	438(97,6)	37(94,9)	0,278	465(97,5)	10(90,9)	0,259
Yes	13(2,7)	0(0)		10(2,3)	3(5,6)		11(2,4)	2(5,1)		12(2,5)	1(9,1)	
Hiccups												
No	468(97,3)	6(85,7)	0,185	422(97,2)	52(96,3)	0,66	438(97,6)	36(92,3)	0,092	464(97,3)	10(90,9)	0,276
Yes	13(2,7)	1(14,3)		12(2,8)	2(3,7)		11(2,4)	3(7,7)		13(2,7)	1(9,1)	
Pain eyes (sensitivity light)												
No	475(98,8)	7(100)	0,999	429(98,8)	53(98,1)	0,507	446(99,3)	36(92,3)	0,999	472(99,0)	10(90,9)	0,128
Yes	6(1,2)	0(0)		5(1,2)	1(1,9)		3(0,7)	3(7,7)		5(1,0)	1(9,1)	
Coma												
No	474(98,5)	6(85,7)	0,109	426(98,2)	54(100)	0,606	443(98,7)	37(94,9)	0,128	470(98,5)	10(90,9)	0,167
Yes	7(1,5)	1(14,3)		8(1,8)	0(0)		6(1,3)	2(5,1)		7(1,5)	1(9,1)	
Confusion												
No	476(99,0)	7(100)	0,999	429(98,8)	54(100)	0,999	445(99,1)	38(97,4)	0,341	472(99,0)	11(100)	0,999
Yes	5(1,0)	0(0)		5(1,2)	0(0)		4(0,9)	1(2,6)		5(1,0)	0(0)	
Bleeding												
No	454(94,4)	7(100)	0,999	407(93,8)	54(100)	0,059	423(94,2)	38(97,4)	0,713	450(94,3)	11(100)	
Yes	27(5,6)	0(0)		27(6,2)	0(0)		26(5,8)	1(2,6)		27(5,7)	0(0)	0,999
Epidemiological link												
No	275(57,2)	3(42,9)	0,597	243(56,0)	35(64,8)	0,289	247(55,0)	31(79,5)	0,011	272(57,0)	6(54,5)	0,463
Yes	45-9,4)	1(14,3)		40(9,2)	6(11,1)		44(9,8)	2(5,1)		44(9,2)	2(18,2)	
Unknown	161(33,5)	3(42,9)		151(34,8)	13(24,1)		158(35,2)	6(15,4)		161(33,8)	3(27,3)	

*Significant difference at 5%.

Table 8. Duration of symptom onset of clinically EVD suspect patients and antibody positivity

Duration (days)	Nucleoprotein		Glycoprotein		40-kDa viral protein		At least two antigens		
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	
Median, 1 st , 3 rd quantiles	3 [2 - 5]								
< 6	382(82.3)	3(42.9)	379(82.9)	36(75.0)	346(83.2)	27(73.0)	355(83.1)	6(54.5)	376(83.0)
6 – 19	76(16.4)	4(57.1)	72(15.8)	10(20.8)	66(15.9)	10(27.0)	66(15.5)	5(45.5)	71(15.7)
> 19	6(1.3)	0(0)	6(1.3)	2(0.1)	4(1.0)	0(0)	6(1.4)	0(0)	6(1.3)
Total	464	7	457	48	416	37	427	11	453

Chapter 2. Longitudinal antibody response in Ebola survivors after specific anti-Ebola treatment

2.1. Introduction

Ebola virus disease (EVD) is a severe viral infection with a mortality rate of up to 90% in the absence of treatment (Sivanandy *et al.*, 2022). The tenth Ebola virus (EBOV) outbreak in the North-Kivu and Ituri provinces was the longest and the deadliest EBOV outbreak ever recorded in the past in the Democratic Republic of the Congo (DRC) and the second largest worldwide following the 2014 West Africa outbreak (Aruna *et al.*, 2019). The outbreak has spiraled out of control as the government's response has been challenged by chronic insecurity caused by the presence of more than a hundred-armed groups, extreme poverty, population displacements, and distrust on the government in the context of general elections organized in the country (Jombart *et al.*, 2020). Kramer *et al.* reported 2,035 conflict events in the DRC between the outbreak declaration on August 1, 2018 and July 26, 2019. Of these, 1,004 conflict events (49%) occurred in the outbreak regions of Ituri and North Kivu (Kraemer *et al.*, 2020). In fact, conflicts and insecurity have seriously hampered the response efforts of the government and other stakeholders.

Considering the risk factors of outbreak extension, several new strategies have been implemented in addition to the traditional EVD outbreaks response measures in order to stop the virus transmission. First, the World Health Organization (WHO) and the Strategic Advisory Group of Experts on Immunization (SAGE) have advised the use of the experimental recombinant vesicular stomatitis virus (rVSV) expressing *Filovirus* glycoprotein (GP) (rVSV-ZEBOV-GP) in a ring vaccination using the expanded access/compassionate protocol (WHO, 2019b). Secondly, experimental specific molecules against Ebola virus were used first in the Monitored Emergency Use of Unregistered and Investigational Interventions (MEURI) (Maxmen, 2019), and after that also in a well-designed randomized clinical trial. A total of four molecules were evaluated, including three monoclonal antibodies (Ansuvimab (also called mAb114), Zmapp and Inmazeb (also called Regeneron) and an antiviral agent (Remdesivir), and among these, two molecules, Inmazeb and Ansuvimab were associated with a high rate of survival (Mulangu *et al.*, 2019).

It is true that the use of specific drugs against the Ebola virus has improved patient care and survival. However, this raises questions that require further investigation. For instance, Ebola virus infection is generally associated with a strong humoral immune response following natural infection, and anti-EBOV immunoglobulin G (IgG) are usually detected from day 6 after the onset of symptoms in non-treated patients. Thereby, the question remains whether the use of monoclonal antibodies and specific antiviral treatment against EBOV may interfere with the endogenous antibody response against the EBOV? Indeed, data related to the evaluation of the effect of such treatments, especially with monoclonal antibodies, on the endogenous humoral immune response are limited. In experimental animal models, Crowe J. et al., have reported that passively immunized BALB/c mice developed impaired humoral immune response after being vaccinated (Crowe *et al.*, 2001). Likewise, other researchers have reported the evidence of the inhibition of the antibody production in vaccinated mice by passively acquired maternal antibodies (Murphy *et al.*, 1991; Siegrist *et al.*, 1998). In addition, a study conducted in confirmed coronavirus disease 19 (COVID-19) human patients has reported suppressed endogenous anti-spike IgM response by 85-90% in patients who were treated with monoclonal antibodies therapies, suggesting a possible damping of natural immune response by passive immunization (Kim *et al.*, 2022).

In DRC, participants that survived from Ebola virus disease, including those from the tenth EVD outbreak in the east, are monitored within the national program of EVD survivors where the follow-up consists in the evaluation of clinical outcomes, biological features and mental psychological support. As such, we identified a cohort of EVD survivors who have been treated with specific EBOV drugs and who have provided biological samples at the follow-up visits. Following the experimental evidences of the effect of monoclonal antibodies on natural immune response, we hypothesized that, patients who have been treated with specific anti-Ebola treatment could develop an impaired antibody response compared to the previous EVD survivors for whom treatment was not yet available.

2.2. Methods

2.2.1. Study design and participants

We conducted a retrospective cohort study from the multicenter longitudinal assessment of Ebola survivors enrolled during the 10th EVD outbreak in North Kivu and Ituri in the DRC under the PNSPG. Previously confirmed and cured Ebola individuals (≥ 5 years) were enrolled in the program after their discharge from the Ebola Treatment Center (ECT) in three operational clinics, Beni, Mangina and Butembo. We included all participant who were followed prospectively after enrollment at months 1, 3, 6, and 12 of follow-up schedule in the program and for whom the information of the acute phase was available. The monitoring covered the period from June 2020 to December 2021. However, the maximal follow-up time for certain patients is equal to three years considering day 0 of discharge from the ETC. Furthermore, the protocol of the study was revised and approved by the ethical committee of the Public Health School (ESP) of the University of Kinshasa (UNIKIN). Participants have provided a written informed consent form before their enrollment in the study.

2.2.2. Data collection and measurements

We used only once-thawed left-over blood samples of participants stored at -80°C in the biobank of the INRB for the samples of the acute phase. The blood samples from the “vainqueurs d’Ebola” project were collected in dry collection tubes and stored at -20°C then sent to the Biobank of the INRB for storage at -80°C until programming serological analysis. We used the national master line list and laboratory database of the tenth Ebola outbreak to collect data recorded during the acute phase of patients with Ebola, and we then used prospectively recorded data during the survivor’s follow-up visits. Data collected included, sociodemographic variables (age, sex, site of enrollment), clinical variables (date of onset of symptoms, date of diagnosis, ETC checkout date, the interval between onset and the day of discharge from ETC, cycle threshold values, and EBOV specific treatment), and laboratory variables (EBOV RT-PCR positivity). The EBOV specific antibody status in EVD survivors were measured using the highly sensitive and specific Multiplex immunoassay (MIA) based on x.MAP[®] (Luminex) Technology (Luminex Corp., Austin, TX) as described previously. The primary outcome was determined by the level of antigen

reactivity at visit M12 in the EVD survivor's cohort expressed as MFI. We have defined as secondary outcome the serology status of survivors at the ETC discharge day (D0). The explanatory (exposure) variables assessed were; specific EBOV molecules received by the survivors during the acute phase of the disease (Ansuvimab (mAb114), Inmazeb (Regeneron), Zmapp, and Remdesivir), we also controlled the effect of age, sex, the interval between onset of symptoms and discharge day from ETC, hospitalization duration, and CT values.

2.2.3. Statistical analysis

Descriptive statistics were performed and expressed in frequencies or proportions for categorical variables while continuous variables were expressed as mean majored by standard deviation or median and percentiles. Chi-square test was used for the comparison of two or more categorical variables while Kruskal-Wallis Test has been performed for the comparison of non-normally distributed continuous variables. For qualitative assessment of seropositivity, antigen's MFI were dichotomously divided into two categories ("positive" and "negative") based on the previously established and revised cut-off value (nucleoprotein positive if MFI > 600; glycoprotein positive if MFI > 400; viral protein 40 positive if MFI > 650) (Diallo *et al.*, 2019). We then performed regression models to evaluate the association between serological status and the different molecules used in the RCT and the MEURI protocol for the treatment of acute EVD. A linear regression model was performed to evaluate the association between specific antibody levels between treatment groups on the day of ETC discharge visit. Furthermore, we assessed longitudinal antibody levels between treatment groups using mixed linear regression model and logistic mixed model. We used *Zaire Ebolavirus* strains *GP-EBOV-mayinga*, *NP-EBOV* and *VP-40 EBOV* antigens and their corresponding MFI and assessed the determinant associated with quantitative evolution of anti-EBOV antibodies over time. Random intercept and slope models were performed using fixed effects (molecules, age, sex and CT values that were recorded during the acute phase of the disease into the model while time and patient identification were used as random effects. For the second model, we have applied positivity criterion to absolute MFI for all tree antigens in order to produce binary variable of positive and negative participants. In the multivariable model, we included molecules, CT value, sex, and age. Finally, we also compared antibody concentration in our cohort with the

outcomes of EVD survivors of the 2014-2016 EVD outbreak in Guinea. The p-value less than 0.05 was used for determination of statistical signification.

2.3. Results

2.3.1. General characteristics of the participants

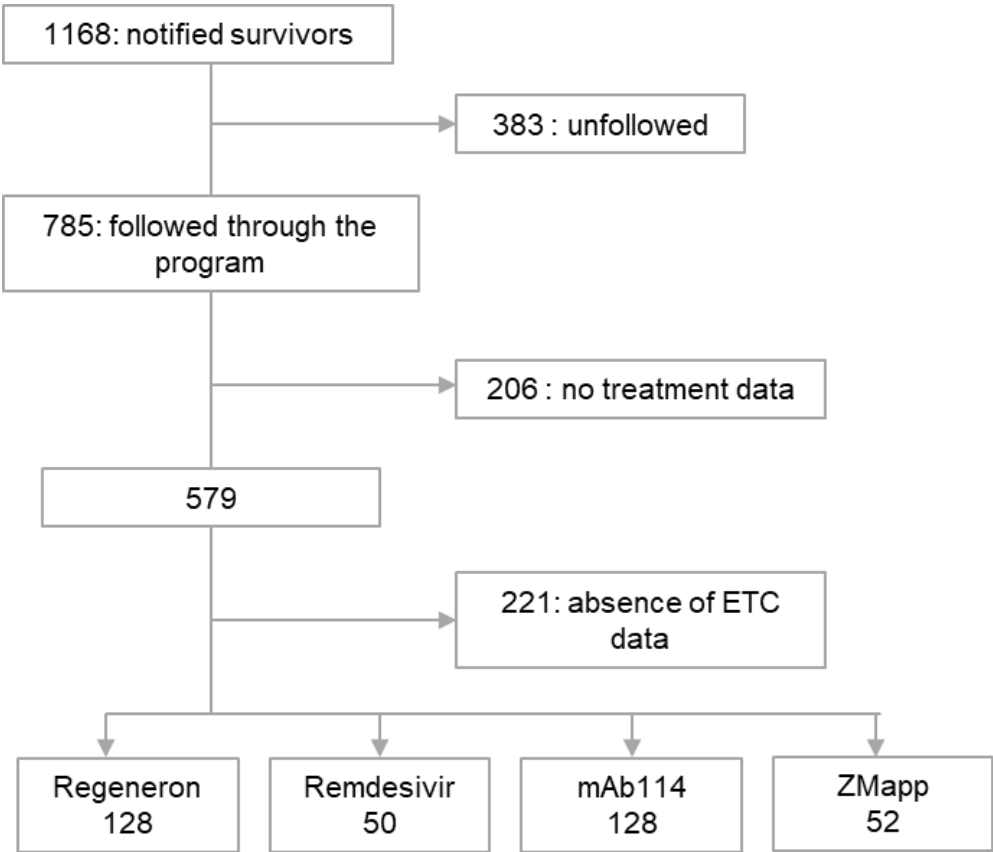


Figure 18. Flowchart of participants inclusion

Of 684 EVD survivors from the 2018-2020 outbreak in eastern Democratic Republic of Congo who were followed for 12 months, 358 participants for whom plasma samples discharged during acute EVD were available were enrolled in the immunologic cohort study (Figure 18). Among them 21 (5.9%) participants provided two blood samples, and 337 (94.1%) provided three or more than three blood samples. A total of 1,421 blood samples were provided and assessed for EBOV antigen positivity by serologic analysis (Table 9). Enrollment started on April 16, 2020 and ended on October 12, 2021.

Table 9. Frequency of blood samples provided and number of tests realized

Blood samples provided	Frequency	Percentage	Number of tests
Two blood samples	21	5,9	42
Three blood samples	139	38,8	417
Four blood samples	68	19,0	272
Five blood samples	90	25,1	450
Six blood samples	40	11,2	240
Total	358	100,0	1421

The overall median age of participants was 29 years (IQR 20 – 42). The median of age was higher in Remdesivir group compared to other treatment groups, and the observed difference was statistically significant ($p < 0.05$). Furthermore, 153 (42.9%) were males and 204 (57.1%) were females. The majority of individuals enrolled were recruited in Beni 153 (42.7%), 140 (39.1%) were from Butembo enrollment site, and 65 (18.2%) came from the Mangina site, and there was no statistically difference between treatment groups. We also reported the median of the interval between onset of symptoms and discharge from ETC of 20 days (IQR 15 – 27). Of 358 participants, 128 (35.8%) were treated with Inmazed, 50 (14.0%) with Remdesivir, 52 (14.5%) with Zmapp, and 128(35.8%) with Ansuvimab (Table 10).

Table 10. Baseline characteristics of participants at the discharge from Ebola Treatment Center

Characteristics	Anti-Ebola Molecules					p-value
	Total n=358	Inmazeb n=128	Remdesivir n=50	Zmapp n=52	Ansuvimab n=128	
Age (years), <i>median (IQR)*</i>	29(20-42)	29(19-40)	35,5(23-48)	30(23-46,5)	28(19-39,5)	<0.001
Sex						0.270
Male, <i>n(%)</i>	153(42,9)	53(35,6)	23(15,0)	28(18,3)	49(32,0)	
Female, <i>n(%)</i>	204(57,1)	74(36,3)	27(13,2)	24(11,8)	79(38,3)	
Visit sites						0.212
Beni, <i>n(%)</i>	153(42,7)	50(32,7)	25(16,3)	24(15,7)	54(35,3)	
Butembo, <i>n(%)</i>	140(39,1)	52(37,1)	19(13,6)	24(17,1)	45(32,1)	
Mangina, <i>n(%)</i>	65(18,2)	26(40,0)	6(9,2)	4(6,2)	29(44,6)	
Cycle threshold values						0.461
≤21	50(14,0)	21(42,0)	6(12,0)	4(8,0)	19(38,0)	
>21	308(86,0)	107(34,7)	44(14,3)	48(15,6)	109(35,4)	
Onset duration (days), <i>median (IQR)</i>	20(15-27)	20(16-27)	20(15-30)	20(16-26)	19(15-26)	0.275
Hospitalization duration (days), <i>median (IQR)</i>	16(12-21)	16(13-21)	17(13-23)	14,5(12,5-18,5)	15(11-19,5)	<0.001

*IQR, Interquartile range

2.3.2. Ebola serology status at the day of discharge as negative by specific RT-PCR from the Ebola treatment center

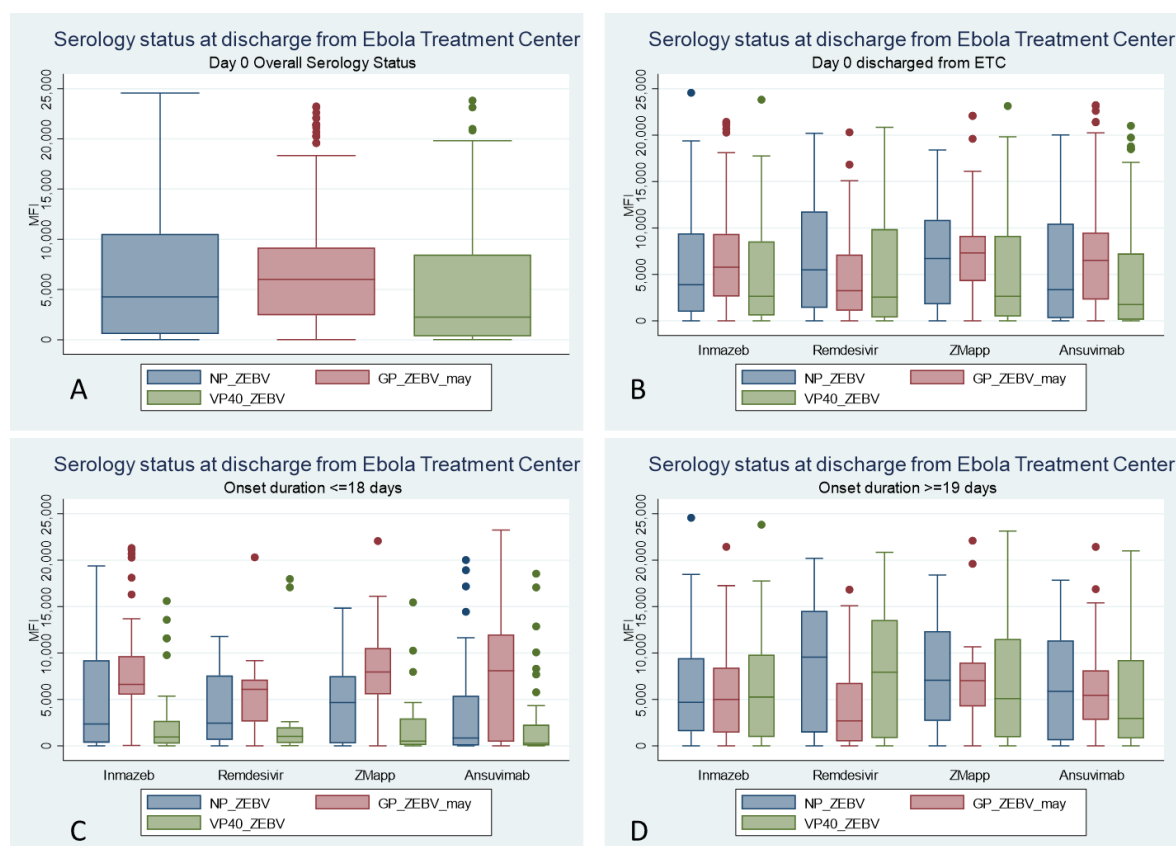


Figure 19. Distribution of anti-Ebolavirus antibodies levels among treatment groups

Kruskal-Wallis Test	Nucleoprotein	Glycoprotein	Viral protein 40
p-value	<0.001	0.152	0.001

Of 358 EVD survivors at day 0, equal to discharge from ETC, 25.4% (91/358) were negative to EBOV-NP, 21.2% (76/358) were negative to EBOV-GP, 26.3% (94/358) were negative to VP40, and 23.7% (85/358) were non-reactive to at least two EBOV antigens. Quantitatively, the overall MFI median for EBOV-NP on discharge day was 4255.8 [IQR (564.5 - 10521)], it was 3884.3 [IQR (976 – 9426.5)] in patients treated with Inmazeb, 5488 [IQR (1405 – 11773)] for the patients who received Remdesivir, 6720 [IQR (1787.5 – 10874.5)] in the Zmapp receiver patients, and 3367.5 [IQR (287.5 – 10460)] for those who have been treated with Ansuvimab. For EBOV-GP, the overall MFI median at day 0 was 6009 [IQR (2438 – 9166)]. However, the calculated EBOV-GP MFI median for patients who received Inmazeb was 5789 [IQR (2632.5 – 9347)], it was 3246 [IQR (1090 – 7145.5)] for the Remdesivir participant group, 7323.6 [IQR (4279.8 – 9166)] for the Zmapp group,

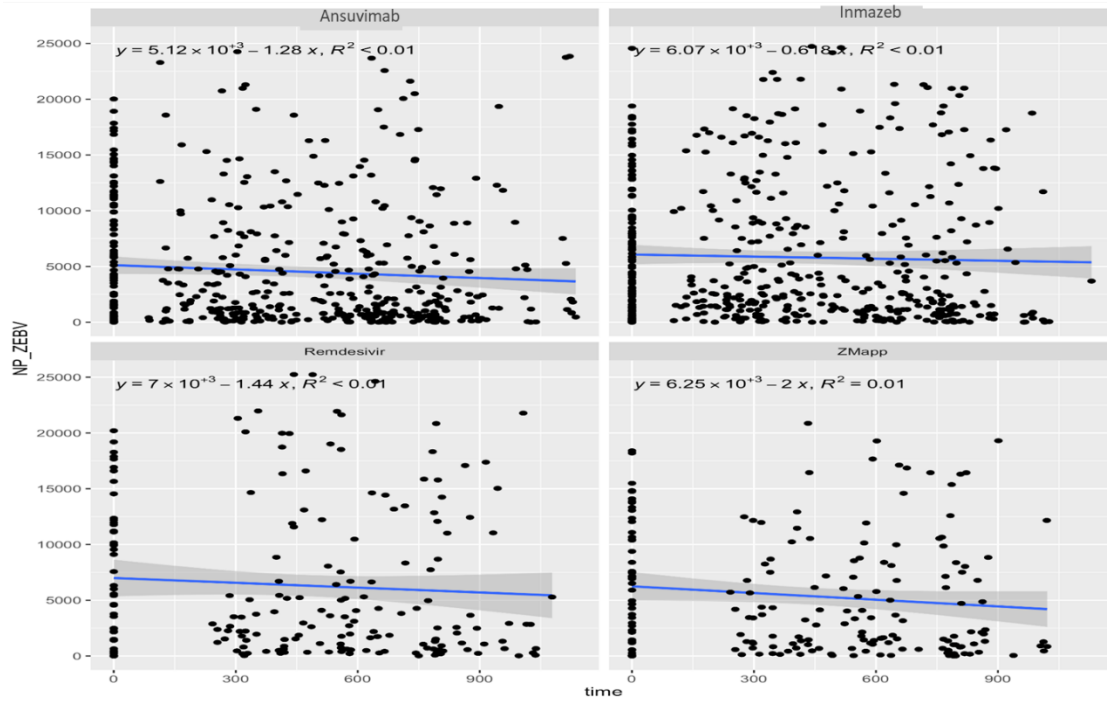
and 6506.8 [IQR (2294 – 9508.8)] for the Ansuvimab participant group. We also computed the EBOV-VP40 MFI between treatment groups; the overall median was 2244.7 [IQR (337 – 8483)], the median was 2647 [IQR (576.5 – 8554.5)] for Inmazeb participant group, 2556 [IQR (362 – 9878.7)] in participants treated with Remdesivir, 2638.5 [IQR (464.5 – 9162.5)] in the Zmapp group, and 1768.5 [IQR (126.5 – 7256.8)] for those who received Ansuvimab as treatment. The NP and VP40 MFI medians were lower in participants treated with Ansuvimab than in other treatment groups ($p < 0.05$) (Table 11; Figure 19). However, there was no association between the level of antibodies and the treatment groups in the linear regression model.

Table 11. Prevalence of anti-Ebola seropositivity among participant treatment groups at the discharge from Ebola Treatment Center

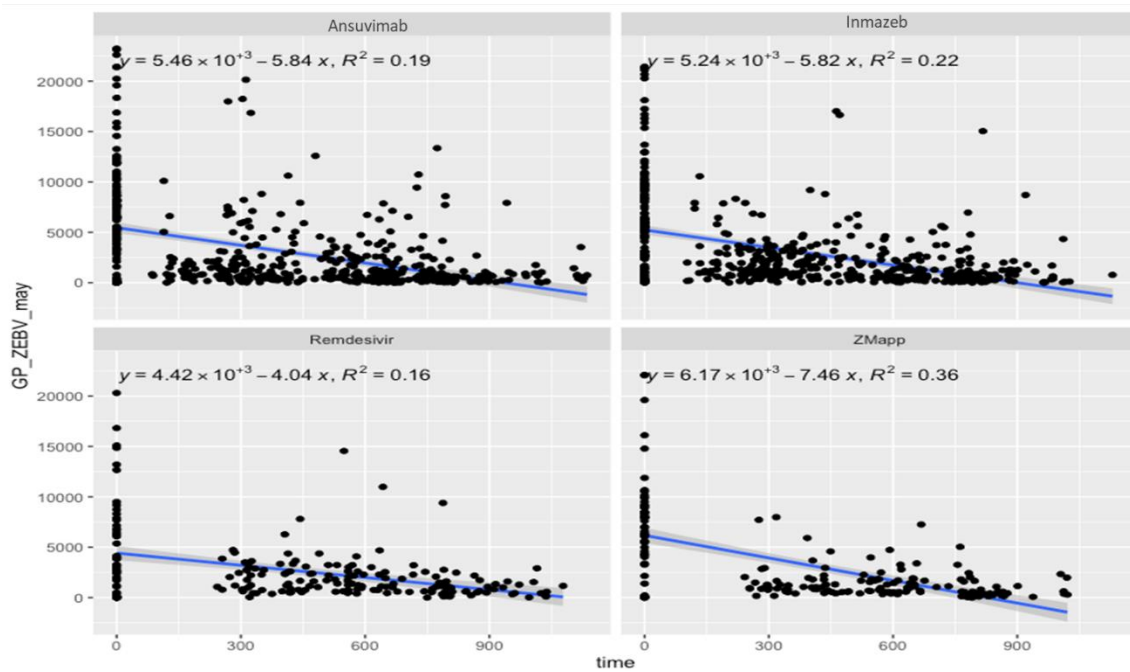
Variables	Anti-Ebola Molecules					p-value
	Total <i>n</i> =358	Inmazeb <i>n</i> =128	Remdesivir <i>n</i> =50	Zmapp <i>n</i> =52	Ansuvimab <i>n</i> =128	
Nucleoprotein						0.058
Positive, n(%)	267(74.6)	100(78.1)	39(78)	43(82.7)	85(66.4)	
Negative, n(%)	91(25.4)	28(21.9)	11(22)	9(17.3)	43(33.6)	
Glycoprotein						
Positive, n(%)	282(80.5)	103(93.0)	41(82)	45(86.5)	93(72.7)	0.153
Negative, n(%)	76(19.5)	25(7.0)	9(18)	7(13.5)	35(27.3)	
Viral protein -40						0.058
Positive, n(%)	264(73.7)	99(77.3)	38(76)	43(82.7)	84(65.6)	
Negative, n(%)	94(26.3)	29(22.7)	12(24)	9(17.3)	44(34.4)	
At least two antigens positives						0.048
Positive, n(%)	273(76.3)	104(81.3)	39(78)	43(82.7)	87(68)	
Negative, n(%)	85(23.7)	24(18.7)	11(22)	9(17.3)	41(32)	

2.3.3. Longitudinal assessment and comparison of anti-Ebola antibodies status among treatment groups

A. Linear evolution for NP



B. Linear evolution for GP



C. Linear evolution for VP

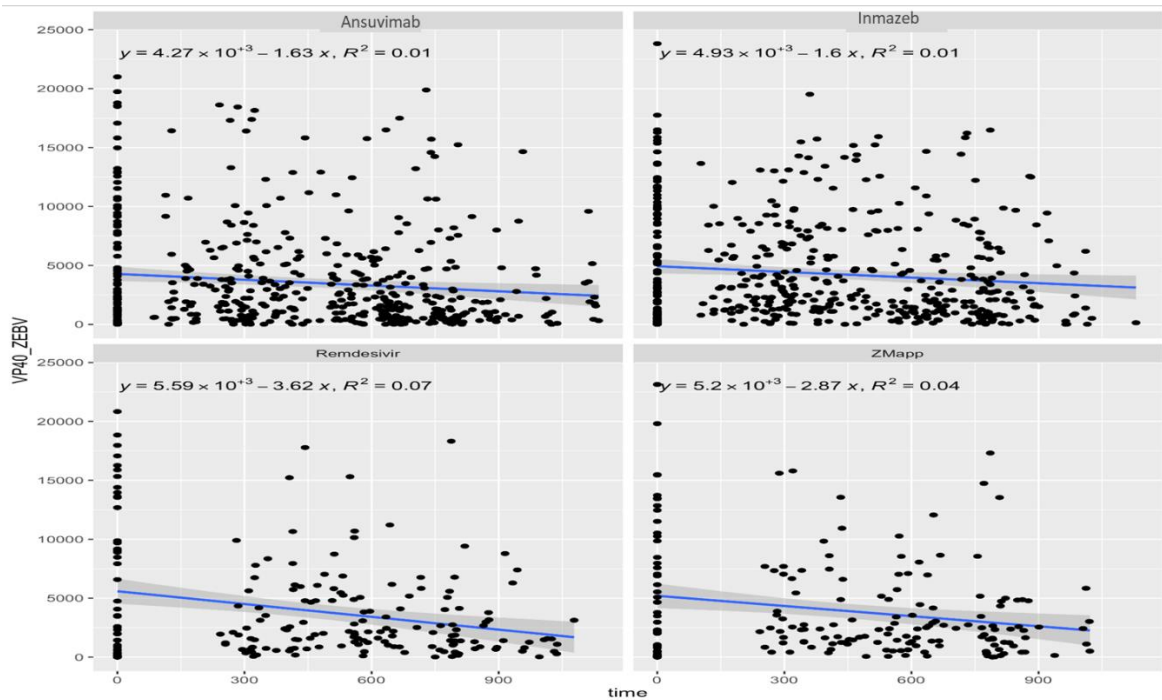


Figure 20. Linear evolution of antibody levels overtime in the four treatment groups.

A) Nucleoprotein, B) Glycoprotein, C) Viral-protein 40.

We measured and compared the level of antibodies to Ebola in survivors of the tenth outbreak of EVD in eastern DRC and found that antibody production in EVD survivors appears to follow a fluctuating evolution over time. Overall, however, the linear evolution showed a continuous decrease in antibody levels overtime and the slope appeared to be steeper for glycoprotein (Figure 20). Using antigens positivity criterion, the positivity level decreased by 4 points (68% to 64%) between the inclusion day and the final day of follow-up for Ansuvimab group, decreased by 6 points (81% to 75%) for Inmazeb group, increased by 3 points (78% to 81%) for Remdesivir group, and decreased by 11 points for participants who received Zmapp treatment (Figure 21).

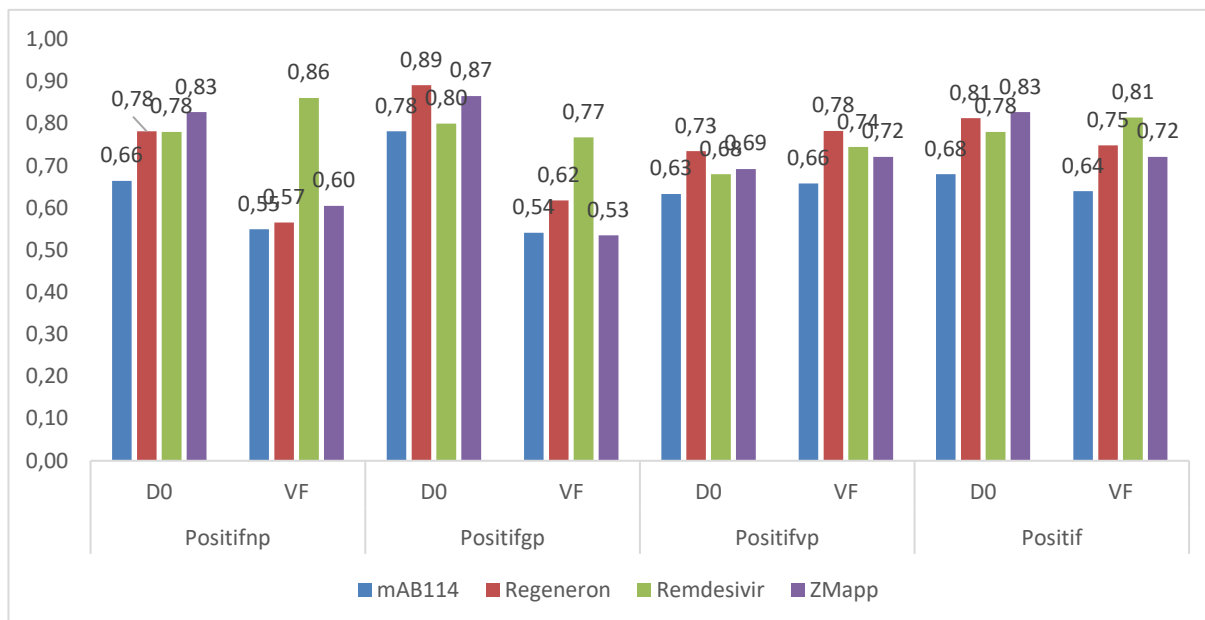


Figure 21. Distribution of the evolution of anti-Ebola antigen positivity among participants over time and by treatment group.

By modeling quantitative concentration of antibody as a function of time, we observed a significant decrease in average of antibody concentration for nucleoprotein ($p < 0.001$), glycoprotein ($p < 0.001$), and viral protein 40 ($p < 0.001$). The decrease seemed to be rapid for antibodies against EBOV glycoprotein. Using the multivariable linear mixed model, we have observed that the linear trend of the time was negative, showing a statistical evidence for the effect of the time in the antibody kinetics cohort (Table 12). For nucleoprotein, the factors at acute phase are associated with higher average of antibody concentration overtime were Inmazeb ($p = 0.003$) and Remdesivir ($p = 0.001$) compared to reference treatment group (Ansuvimab). However, the factors that were associated with lower average of antibody against nucleoprotein were Ct value ($p < 0.001$) and being male ($p = 0.016$) compared to female. From modeling outcomes for viral protein, only Ct value ($p < 0.001$) was associated with the lower average of antibody concentration. For glycoprotein, we did not observe any significant association between antibody concentration and molecules, Ct value, sex, and age ($p > 0.05$). We have used the logistic mixed model to assess the probability of being positive according to the time after being discharged from Ebola treatment center. In

comparison with Guinea survivors, those in our cohort have shown a lower probability of being positive at the discharge time, but also after a total of 36 months of follow-up (Figure 22). For nucleoprotein, the probability of being positive at 36 months was 70.8% (68.9-72.8) for Ansuvimab, 81.4% (79.4-83.4) for Remdesivir, 83.9% (81.6-85.6) for Inmazeb, 87.8% (85.8-89.8) for ZMapp, and 95.6% (91.0-97.9) for Postebogui (Table 13). For viral protein 40, the probability of being positive at 36 months was 54.1% (52.1-56.1) for Ansuvimab, 68.3% (66.3-70.3) for Remdesivir, 69.2% (67.2-71.2) for ZMapp, 75.3% (73.3-77.3) for Inmazeb, and 89.2% (85.1-92.2) for Postebogui (Table 14). For glycoprotein, the probability was 35.2% (33.2-37.2) for Ansuvimab, 45.2% (43.2-47.2) for Inmazeb, 50.0% (48.1-52.1) for ZMapp, 65.7% (63.7-67.7) for Remdesivir, and 92.8% (89.2-95.3) for Postebogui (Table 15). The probability of being positive to at least two EBOV antigens was 53% for mA114, 73% for Inmazeb, 76% for Remdesivir, and 78% for Zmap. Indeed, at the same periode of the time and using the same criteria, the probability was 95% in POSTEBOGUI survivors (Table 16).

Table 12. Association between EBOV antibody concentration and factors in the acute phase of the disease, based on linear mixed model

	NP-EBOV		VP40-EBOV		GP-EBOV-m	
	<i>Estimate</i>	<i>p value</i>	<i>Estimate</i>	<i>p value</i>	<i>Estimate</i>	<i>p value</i>
Time*. days	-0.9(-1.9 to -0.05)	0.039*	-1.949(-2.6 to -1.3)	<0.001***	-5.7(-6.3 to -5.1)	<0.001***
Treatments						
Ansuvimab	Reference	..	Reference	..	Reference	..
Inmazeb	1018(326 to 1709)	0.003**	487(-26 to 1001)	0.062	-238(-662 to 184)	0.269
Remdesivir	1532(605 to 2460)	0.001**	264(-425 to 954)	0.451	-415(-983 to 152)	0.152
ZMapp	861(-77.3 to 1800)	0.072	430(-267 to 1128)	0.227	27(-547 to 602)	0.925
Sex						
Female	Reference	..	Reference	..	Reference	..
Male	-737(-1340 to -133)	0.016*	-5.5(-454 to 443)	0.980	87(-281 to 457)	0.641
Ct value	-348(-406 to -291)	<0.001***	-212(-255 to -169)	<0.001***	-4.1(-39.4 to 31.1)	0.818
Age	-1.7(-20 to 16)	0.861	-10.07(-23 to 3.7)	0.150	-3.36(-14.7 to 7.9)	0.561

Data are median fluorescence intensity per 100 beads (95% CI). NP-EBOV=nucleoprotein *Ebolavirus* mayinga strain 1976. VP40-EBOV=viral protein *Ebolavirus* mayinga strain 1976. GP-EBOV=glycoprotein *Ebolavirus* mayinga strain 1976. Time since discharge from Ebola Treatment Center.

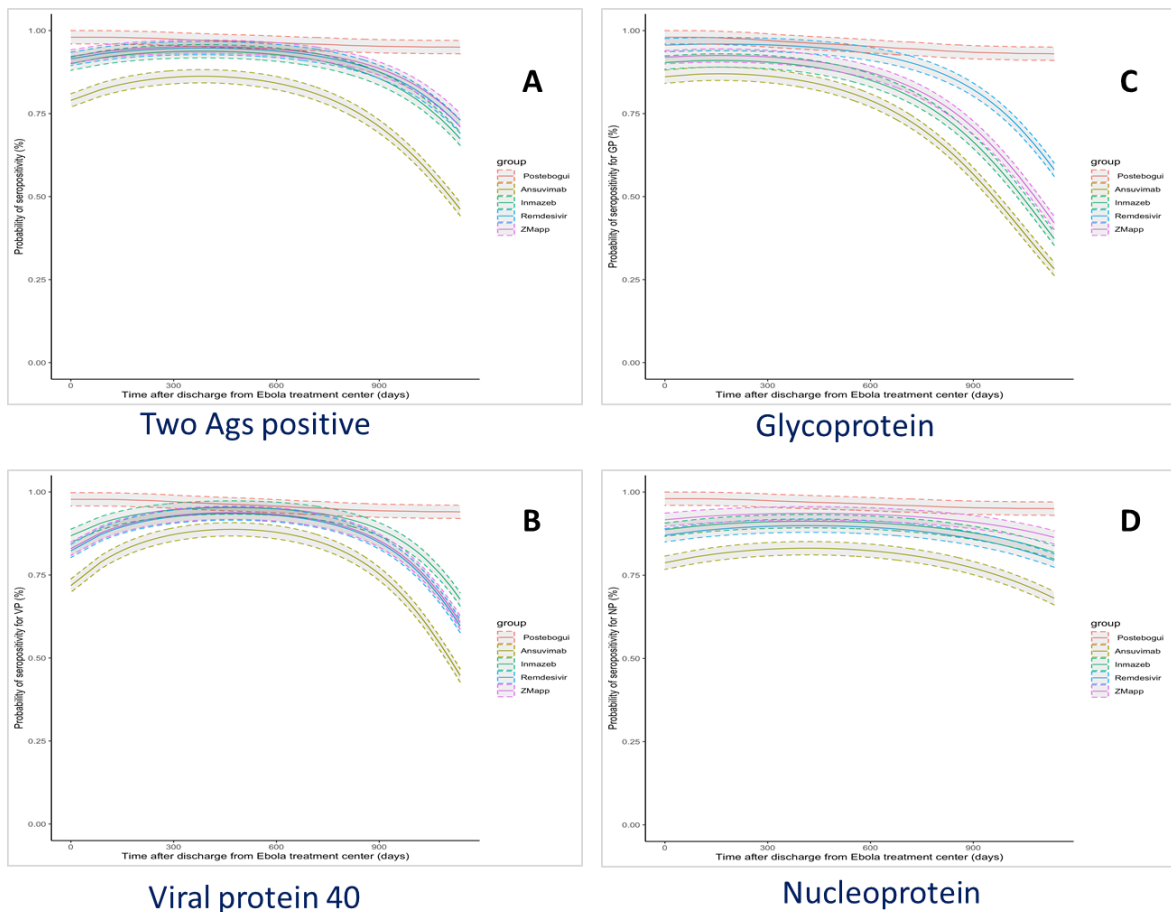


Figure 22. Probability of being positive to Ebola antigens among survivors who received specific treatment according to time after discharge from the Ebola treatment Center.

For positivity criteria presence of antibodies to at least two antigens (A), to VP 40 (B), to GP (C) and to NP (D), compared to observations of non-treated patients from the PostEbogui study in guinea.

Table 13. Probability of Seropositivity for Nucleoprotein over time among Ebola survivors

Months after discharge from ETC*	Nucleoprotein				
	Inmazeb	Remdesivir	Zmapp	Ansuvimab	Postebogui
6 months	90.46% (88.46-92.46)	89.01% (87.01-91.01)	93.01% (91.01-95.01)	81.77% (79.77-83.77)	99.82% (98.82-99.92)
12 months	91.19% (89.19-93.19)	89.84% (87.84-91.84)	93.56% (91.56-95.56)	83.04% (81.04-85.04)	99.80% (98.85-99.93)
24 months	89.96% (87.96-91.96)	88.44% (86.44-90.44)	92.63% (90.63-94.63)	80.90% (78.90-82.90)	98.85% (96.75-99.59)
36 months	83.96% (81.69-85.69)	81.43% (79.43-83.43)	87.82% (85.82-89.82)	70.82% (68.82-72.82)	95.67% (91.05-97.97)

*ETC: Ebola Treatment Center

Table 14. Probability of Seropositivity for Viral protein 40 over time among Ebola survivors

Months after discharge from ETC*	Viral protein 40				
	Inmazeb	Remdesivir	Zmapp	Ansuvimab	Postebogui
6 months	92.96% (90.96-94.96)	90.31% (88.31-92.31)	90.70% (88.70-92.70)	83.65% (81.65-85.65)	98.57% (96.57-99.87)
12 months	95.04% (93.04-97.04)	93.11% (91.11-95.11)	93.39% (91.39-95.39)	88.12% (86.12-90.12)	97.79% (95.95-98.79)
24 months	93.69% (91.69-95.69)	91.29% (89.29-93.29)	91.64% (89.64-93.64)	85.18% (83.18-87.18)	95.02% (92.25-96.84)
36 months	75.34% (73.34-77.34)	68.31% (66.31-70.31)	69.27% (67.27-71.27)	54.18% (52.18-56.18)	89.23% (85.19-92.27)

*ETC: Ebola Treatment Center

Table 15. Probability of Seropositivity for Glycoprotein over time among Ebola survivors

Months after discharge from ETC*	Glycoprotein				
	Inmazeb	Remdesivir	Zmapp	Ansuvimab	Postebogui
6 months	91.00% (89.00-93.00)	95.92% (93.92-97.92)	92.49% (90.49-94.49)	86.97% (84.97-88.97)	98.71% (96.71-99.91)
12 months	89.98% (87.98-91.98)	95.43% (93.43-97.43)	91.63% (89.63-93.63)	85.57% (83.57-87.57)	98.15% (96.32-99.08)
24 months	80.02% (78.02-82.02)	90.31% (88.31-92.31)	91.63% (89.63-93.63)	85.57% (83.57-87.57)	98.15% (96.32-99.08)
36 months	45.22% (43.22-47.22)	65.77% (63.77-67.77)	50.01% (48.16-52.16)	35.28% (33.28-37.28)	92.87% (89.25-95.34)

*ETC: Ebola Treatment Center

Table 16. Overall probability of seropositivity over time among Ebola survivors

Months after discharge from ETC	At least two antigens positive				
	Inmazeb	Remdesivir	Zmapp	Ansuvimab	Postebogui
6 months	92.88% (90.88-94.88)	93.95% (91.95-95.95)	94.48% (92.48-96.48)	84.43% (82.43-86.43)	99.82% (99.82-99.92)
12 months	93.78% (91.78-95.78)	94.71% (92.71-96.71)	95.18% (93.18-97.18)	86.23% (84.23-88.23)	99.50% (98.67-99.81)
24 months	91.06% (89.06-93.06)	92.37% (90.37-94.37)	93.03% (91.03-95.03)	80.88% (78.88-82.88)	98.50% (98.18-98.97)
36 months	73.52% (71.52-75.52)	76.76% (74.76-78.76)	78.45% (76.45-80.45)	53.57% (51.57-55.57)	95.58% (93.06-96.62)

*ETC: Ebola Treatment Center

2.4. Discussion

Here we reported a longitudinal assessment of antibody kinetics in EVD survivors treated with specific newly developed molecules during the 2018-2020 EVD outbreak that occurred in eastern DRC. Each participant received one of four experimental molecules during the acute phase, in an emergency MEURI protocol or in a rigorous randomized clinical trial (Maxmen, 2019; Mulangu *et al.*, 2019). Overall, 23.7% were seronegative for at least two antigens at discharge from the ETC endpoint. Quantitatively, NP and VP40 MFI were lower in participants treated with Ansuvimab. In fact, EBOV is a fatal disease that often induces a strong immune response in the infected host within days of disease onset, that is reported to be between eight and ten days (Ksiazek, Rollin, *et al.*, 1999; Tshiani Mbaya *et al.*, 2021). The median of the interval between the onset of symptoms and the discharge day from ETC was 20 days in this cohort. Although a few studies have reported suppression of the endogenous immune response by passive immunization (Crowe *et al.*, 2001; Kim *et al.*, 2022; Murphy *et al.*, 1991), we cannot conclusively conclude that this is the case in our cohort. However, these results contribute to a good understanding of antibody production during the acute phase of EVD. Our cohort is, to our knowledge, the first to comprehensively assess the immune response against EBOV in a large cohort of survivors at the time discharged from treatment centers and after specific Ebola treatment. Furthermore, we cannot exclude the possible dampening of the immune response by the action of monoclonal antibodies on the virus, which prevents the fusion between the viral glycoprotein and the host membrane, and the antiviral molecule which inhibits the replication of the virus (Sivanandy *et al.*, 2022).

In the individual survivors, the antibody trend showed an up-and-down trend, while we observed a continuous decrease in an overall linear evolution. In some patients however, we observed an increase of average-antibody concentration over time. This result has been reported in studies prior to ours (Adaken *et al.*, 2021; Diallo *et al.*, 2021). As a hypothesis, in fact, the continuous stimulation linked to the probable persistence of the virus in immune privileged sites has been advanced. In the study from Guinea, Diallo *et al.*, the persistence of the virus in semen was associated with increased antibody concentrations (Diallo *et al.*, 2021). However, the up-and-down antibody kinetics cannot be explained only by the viral persistence. In fact, the innate response at the acute phase as well as the robust and

polyfunctional EBOV-specific T-cell response also play an important role in the clearance of the virus and the activation of memory B-cells clones (Wiedemann *et al.*, 2020). We have observed that the treatment factors in the acute phase that were significantly associated with higher average of antibody concentration to nucleoprotein were Inmazed, ZMapp, and Remdesivir compare to Ansuvimab. Overall decrease in antibody concentration was observed in these participants. In fact, monoclonal antibody-based treatment used during the epidemic did not target EBOV nucleoprotein while we cannot exclude the possible interaction with these molecules with endogenous process of innate immune activating adaptive immune system (MacIntyre *et al.*, 2016). Antibody levels for glycoprotein were higher in participants treated by Inmazed and Remdesivir compare to those who received Ansuvimab while the association was not significant. Furthermore, we have reported a rapid decline in antibody against glycoprotein concentration over time. This finding is in contrast with the report from Guinea where antibody against nucleoprotein likely declined faster compare to those induced against viral protein 40 and glycoprotein of *Ebolavirus* (Diallo *et al.*, 2021). This may be explained by the fact that passive immunity targeting the EBOV glycoprotein can interact with the epitope-specific memory B cell clones of the glycoprotein, resulting in partial impairment of the anti-glycoprotein humoral immune response in patients received mAbs. Indeed, passive immunization has been documented to suppress the humoral immune response in mice vaccinated with the live attenuated respiratory syncytial virus (RSV) vaccine (Crowe *et al.*, 2001). Therefore, a risk factor for EVD relapse or reinfection over time may be due to the rapid fall of anti-EBOV antibodies in EVD survivors who have received treatment. For instance, two non-human primates treated with monoclonal antibodies after an initial EBOV infection died when they were experimentally reinfected with the virus. Subsequent investigation showed that they had a weak humoral response to the initial infection (MacIntyre *et al.*, 2016; Qiu *et al.*, 2013). Thus, if the viral load during reinfection is important and the patient has lost his antibodies quickly, it is theoretically possible that an EVD survivor who has totally eradicated the virus will become infected again in the future.

The one unit increase in Ct value in the acute phase was associated with the decrease in mean antibody concentration over time for nucleoprotein and viral protein 40 ($p < 0.001$). The CT value at the time of diagnosis can be considered as a reflection of the severity of clinical Ebola virus disease. Patients with a low Ct are thought to have a high viral load and

are at risk of developing severe EVD. The severity of the disease is characterized by the appearance of symptoms of the so-called wet phase. In survivors from Guinea, vomiting was associated with low-average anti-nucleoprotein antibodies (Diallo *et al.*, 2021). However, the cytokine storm seen in EVD is more prevalent in patients with a high viral load and is generally associated with overexpression of genes that encode for IFN synthesis (Wiedemann *et al.*, 2020). Indeed, inflammation in survivors of Ebola virus disease appear to be lower compared to fatal cases, in whom the overexpression of cytokine appears to be correlated with high viral load (Colavita *et al.*, 2019). However, in our study, high viral load was associated with high average antibody concentration for the nucleoprotein. It cannot be excluded that this is due to the fact that all patients received specific treatment. We have also calculated the probability of being positive to EBOV antigens among four treatments groups and we noted that the curves decrease as a function of time, but this decrease appears to be rapid for the Ansuvimab-treated patient group. In addition, all curves decrease rapidly compared to the curve of EVD survivors followed from Guinea.

The present study is the first that evaluated the temporal kinetics of Ebola virus antibodies in a cohort of survivors, all treated with Ebola virus-specific molecules. Indeed, we observed that nearly 25% of patients were negative for at least two antigens at the exit of ETCs. This information may have direct implications for the treatment of people discharged from ETCs, where it may be possible to vaccinate survivors to stimulate memory lymphocytes, responsible for antibody production. We also observed that survivors who received specific treatments seem to have a faster fall in antibody concentration than in the group of untreated survivors, and that this fall seems to be faster in the group of survivors treated with Ansuvimab. Indeed, Ansuvimab induces virus neutralization by targeting an essential step in the mechanism of virus entry into the cell by recognizing the epitope spanning both the glycan cap and the GP1 core, thereby reducing exposure to RBD by protease cleavage and receptor binding prevention (Misasi *et al.*, 2016). Although other monoclonal antibodies used as therapy do not recognize the same glycoprotein epitope as Ansuvimab. It is therefore necessary to clarify how Ansuvimab can be associated with rapid degradation of antibodies over time. Recently, an unknown EVD case was the index case during the 2021 EVD epidemic in Guinea (Keita *et al.*, 2021). EVD relapse cases reported in the DRC demonstrated also the likely role of the human reservoir in the emergence of Ebola (Mbala-Kingebeni *et al.*, 2021). Thus, these findings are important in the way that

they emphasize the need to continue research on antibody kinetic in Ebola survivors but also evaluate the implication of specific treatment in order to improve care for EBOV survivors and propose appropriate public health measures.

Our study has several limitations. First, our detection tools were unable to distinguish antibodies provided as therapy from endogenous antibodies at the endpoint of CTE release. Therefore, monoclonal antibodies provided to patients may have interfered with the measurement of antibodies to EBOV glycoprotein at this endpoint. Some confirmed EVD cases were listed as confirmed contact cases before they developed infection and they may have been vaccinated. Unfortunately, we lacked information on vaccination status and this may also interact with the measurement of anti-glycoprotein antibodies. Furthermore, being positive for EBOV antibodies does not mean having neutralizing antibodies. Here we have not evaluated the neutralization of the binding antibodies that we have detected, but we have also not characterized the IgG isotypes.

2.5. Conclusion

In conclusion, this is the first study at our knowledge that has evaluate the temporal antibody kinetic for up to 36 months among EVD survivors treated with specific anti-Ebola molecules. We were able to show that around 25% of survivors were negative to at least two antigens at the discharge from ETC and the individual trend of antibody was up-and-down but with an overall global linear decrease of antibody concentration over time. Treated survivors have shown to have an overall rapid decrease of antibody over time in comparison with an untreated cohort of survivors. We also have observed a rapid decrease of antibody concentration in participants who received Ansuvimab at the acute phase of the illness. However, we cannot say with certainty that these observations are due solely to the treatments received. It is therefore very useful to continue our research on this question to better understand the observations made.

2.6. Ebola Virus Transmission Initiated by Systemic Ebola Virus Disease Relapse

P. Mbala-Kingebeni. C. Pratt. M. Mutafali-Ruffin. M.G. Pauthner. F. Bile. **A. Nkuba-Ndaye**. A. Black. E. Kinganda-Lusamaki. M. Faye. A. Aziza. M.M. Diagne. D. Mukadi. B. White. J. Hadfield. K. Gangavarapu. N. Bisento. D. Kazadi. B. Nsunda. M. Akonga. O. Tshiani. J. Misasi. A. Ploquin. V. Epaso. E. Sana-Paka. Y.T.T. N'kassar. F. Mambu. F. Edidi. M. Matondo. J. Bula Bula. Diallo. M. Keita. M.R.D. Belizaire. I.S. Fall. A. Yam. S. Mulangu. A.W. Rimion. E. Salfati. A. Torkamani. M.A. Suchard. I. Crozier. L. Hensley. A. Rambaut. O. Faye. A. Sall. N.J. Sullivan. T. Bedford. K.G. Andersen. M.R. Wiley. S. Ahuka-Mundeke. and J.-J. Muyembe Tamfum

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Mbala-Kingebeni at Institut National de Recherche Biomédicale, 5345, Ave. de la Démocratie, B.P. 1187 Gombe, Kinshasa, Democratic Republic of Congo, or at mbalaplacide@gmail.com; or to Dr. Wiley at 984388 Nebraska Medical Center, Omaha, NE 68198-4388, or at mike.wiley@unmc.edu.

Dr. Mbala-Kingebeni, Ms. Pratt, and Drs. Mutafali-Ruffin, Pauthner, Bile, Nkuba-Ndaye, and Black and Drs. Sullivan, Bedford, Andersen, Wiley, Ahuka-Mundeke, and Muyembe Tamfum contributed equally to this article.

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2.6.1. Summary

During the 2018–2020 Ebola virus disease (EVD) outbreak in North Kivu province in the Democratic Republic of Congo, EVD was diagnosed in a patient who had received the recombinant vesicular stomatitis virus–based vaccine expressing a ZEBOV glycoprotein (rVSV-ZEBOV) (Merck). His treatment included an Ebola virus (EBOV)–specific monoclonal antibody (Ansuvimab), and he recovered within 14 days. However, 6 months later, he presented again with severe EVD-like illness and EBOV viremia, and he died. We initiated epidemiologic and genomic investigations that showed that the patient had had a relapse of acute EVD that led to a transmission chain resulting in 91 cases across six health zones over 4 months.

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2.6.2. Introduction

Human-to-human transmission of Ebola virus (EBOV) typically occurs through direct contact with infectious blood or bodily fluids (e.g. semen or breast milk).¹ EBOV persistence has been well documented in survivors of Ebola virus disease (EVD). Although secondary transmission through contact with infectious bodily fluids is relatively rare, it has been linked to flare-up events.² In two survivors of EVD, meningoencephalitis and uveitis syndromes have been associated with infectious EBOV in cerebrospinal fluid and aqueous humor, respectively; neither case led to further transmission.^{3,4} Here, we report the relapse of acute EVD in a patient who was infected with the EBOV Ituri variant during the 2018–2020 North Kivu EVD outbreak in the Democratic Republic of Congo (DRC).⁵ The relapse, which occurred 149 days after the patient was discharged from an Ebola treatment unit, sparked a transmission chain of 91 cases.

2.6.3. Case report

Patient history and epidemiologic findings

A 25-year-old man who worked as a motorcycle taxi driver presented to the Ebola treatment unit (ETU) in Mangina, DRC, on June 15, 2019, with a 2-day history of fever, nausea, vomiting, asthenia, anorexia, myalgia, and chest pain. According to the patient's medical record, he had received the recombinant vesicular stomatitis virus–based vaccine expressing a ZEBOV glycoprotein (rVSV-ZEBOV)⁶ (Merck, lot number WL00064825) 6 months previously (on December 6, 2018) because he was a contact of a person with confirmed EVD. Despite his previous vaccination, we detected EBOV RNA in the patient's serum with the use of the GeneXpert platform (Xpert Ebola Assay, Cepheid) (Table 17), and EVD was diagnosed.

Table 17. Diagnostic Test Results in Samples Obtained during the First and Second Episodes of EVD in the Patient in 2019*

Sample and Laboratory Identifiers†	Date Sample Collected	Sample Type	Glycoprotein Ct Value	Nucleoprotein Ct Value	Glycoprotein IgG EC ₅₀ Titer	Virus Sequenced‡
Sample d1: MAN4194	June 15	Serum	32.5	29.9	Negative	Yes
MAN4337	June 18	Serum	Negative	41.7	—	—
MAN4434	June 20	Serum	41.3	39.2	—	—
MAN4524	June 22	Serum	Negative	38.5	—	—
MAN4694	June 25	Serum	Negative	38.0	—	—
MAN4796	June 27	Serum	Negative	Negative	—	—
Sample d14: MAN4907	June 29	Serum	Negative	Negative	1:77.579	—
Identifier unknown	Aug. 27	Semen	Negative	Negative	—	—
Sample d171: MAN12309	Dec. 3	Serum	33.3	30.1	1:164.609	Yes
Sample d173: MAN12369	Dec. 5	Oral swab	28.7	24.8	—	Yes

* The glycoprotein and nucleoprotein targets of Ebola virus RNA were detected with the use of GeneXpert diagnostic quantitative reverse-transcriptase-polymerase-chain-reaction assays (Xpert Ebola Assay, Cepheid) and are expressed as cycle-threshold (Ct) values. Glycoprotein binding titers were assessed with the use of an enzyme-linked immunosorbent assay (Alpha Diagnostic International) with a readout for the anti-Ebola glycoprotein IgG EC₅₀ (the concentration at which there is a 50% decrease in antigen binding). EVD denotes Ebola virus disease.

† Sample identifiers were assigned only to the samples described in this article.

‡ Samples from which full viral genomes were determined are indicated.

We initiated treatment with the experimental Ansuvimab monoclonal antibody⁷ on June 16, 2019, under the Monitored Emergency Use of Unregistered and Investigational Interventions protocol.⁸ In addition, we provided standard supportive care that included an antibiotic agent (intravenous ceftriaxone), an antimalarial agent (artesunate amodiaquine), a proton-pump inhibitor (omeprazole), and magnesium supplementation. The patient was discharged from the ETU on June 29 after two consecutive negative results on a polymerase-chain-reaction assay (Figure 23 and Table 17). On August 27, a semen sample was collected as part of the national program to monitor EVD survivors, and the test result was negative for EBOV RNA (Table 17). The patient did not undergo follow-up for additional semen testing. On November 25, 2019, a total of 149 days after discharge from the ETU, headache, asthenia, myalgia, polyarthralgia, and anorexia developed in the patient. He was seen at a local health center, where he received unspecified treatment. On November 26, he had nausea, diarrhea, melena, jaundice, conjunctival injection, epistaxis, and abdominal, chest, and spinal pain. The patient consulted a traditional practitioner, was hospitalized for 2 days, and received unspecified treatment. After the symptoms increased in severity, community members alerted the EVD response team, and 8 days after the onset of illness (on December 3), the patient was transferred to the ETU in Mangina. He had

transient loss of consciousness soon after arrival. Initial clinical examination revealed a bedridden patient with pale palpebral conjunctiva, icteric bulbar conjunctiva, a soft abdomen with epigastric tenderness, swelling of the left arm, and tender ecchymosis and bleeding at a venipuncture site. The vital signs on arrival included a heart rate of 91 beats per minute, respiratory rate of 26 breaths per minute, and blood pressure of 100/60 mm Hg ; the oxygen saturation as measured by pulse oximetry was 99%. The results of rapid diagnostic tests to detect human immunodeficiency virus (HIV) infection (Determine, Abbott) and malaria were negative. A serum sample obtained on December 3 tested positive for EBOV RNA (Table 17), and he received a diagnosis of EVD for the second time in 6 months.

The patient received antibiotics (ceftriaxone, metronidazole, and amoxicillin–clavulanate), an antimalarial agent (artesunate–amodiaquine), a proton-pump inhibitor (omeprazole), an anti-emetic agent (ondasetron), and rehydration fluid. On December 4, 2019, his condition deteriorated, with loss of consciousness, gingivorrhagia, anemia, and dyspnea associated with painful hepatomegaly on palpation. Clinical laboratory tests revealed acute kidney injury, liver injury (elevated hepatic aminotransferase levels and hyperbilirubinemia), hyponatremia, severe hypoalbuminemia, and a markedly elevated C-reactive protein level; all these findings were consistent with multiorgan failure or dysfunction (Table 18 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

The patient received oxygen and a blood transfusion. Despite this treatment, acute respiratory distress and coma developed, and the patient died. A postmortem oral swab obtained on December 5, 2019, was positive for EBOV RNA (Table 17).

An epidemiologic investigation was initiated. Contact tracers connected the patient to 29 persons with EVD who had had exposure to the patient while he was symptomatic in the community or while he had visited local health clinics for treatment. An additional 62 cases resulted from onward transmission from these initial contacts. Overall, the cases spanned six health zones over 4 months.

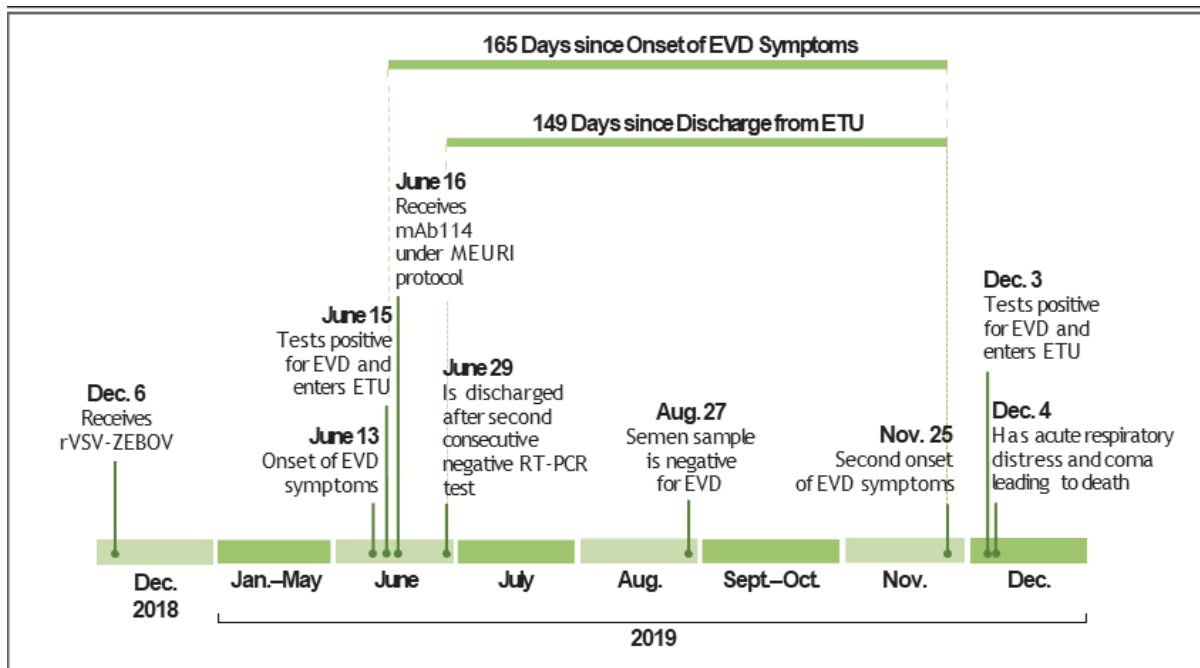


Figure 23. Timeline of the First and Second Episodes of EVD in the Patient, from December 2018 through December 2019.

ETU denotes Ebola treatment unit, EVD Ebola virus disease, MEURI Monitored Emergency Use of Unregistered and Investigational Interventions, RT-PCR reverse transcriptase-polymerase chain reaction, and rVSV-ZEBOV recombinant vesicular stomatitis virus-based vaccine expressing a ZEBOV glycoprotein.

Molecular and serologic investigations

We initiated a genomic investigation to support the epidemiologic findings and differentiate between relapse and reinfection in this patient (i.e. to differentiate between recurrence of the initial disease that had occurred in June 2019 and reinfection through an active transmission chain in November 2019). We sequenced serum samples obtained during the first infection on June 15 (sample d1) and during the second infection on December 3 (sample d171), as well as a postmortem oral swab (sample d173). We also sequenced diagnostic samples obtained from 72 epidemiologically linked cases (Table 19). Our comparison of these samples with previously sequenced samples from the North Kivu outbreak revealed that all our patient's samples (samples d1, d171, and d173) and those from the 72 epidemiologically linked cases shared a unique mutation in the glycoprotein (G6800A/E258K) that separated these sequences from other sequences in the outbreak (Figure 25A). The samples obtained during the second infection (samples d171 and d173) and those from the 72 epidemiologically linked cases shared two unique mutations (T5578C

non- coding and glycoprotein A6867G/E280G) that genetically linked the cluster and indicated that the sample d1 sequence was ancestral to the relapse cluster (Figure 24A and Figure 25B).

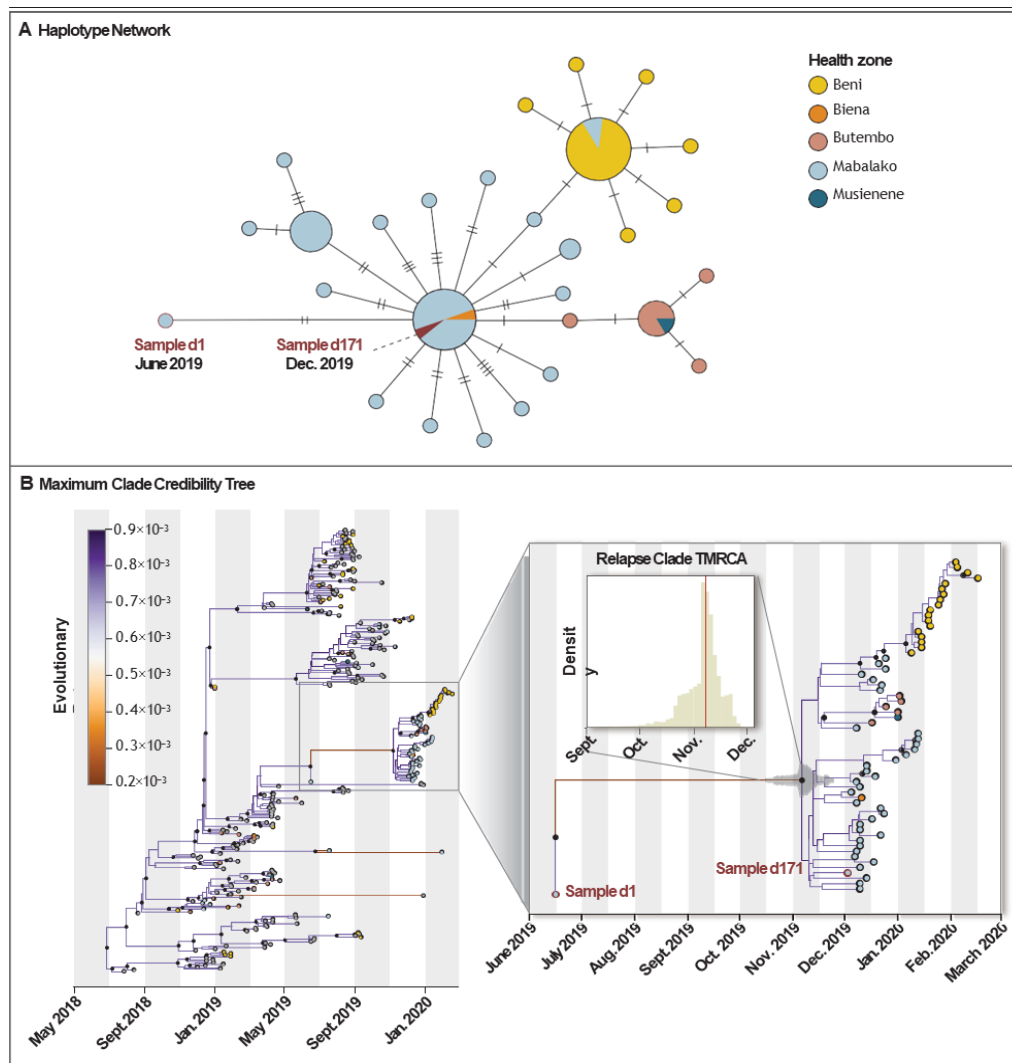


Figure 24. Epidemiologic and Phylogenetic Analysis of the Case of Relapse and Linked Cases.

We initiated investigations that showed that the patient had had a relapse of acute Ebola virus disease (EVD) that led to a transmission chain resulting in 91 cases across six health zones in the Democratic Republic of Congo (DRC). Panel A shows a haplotype network of the case of EVD relapse and 72 epidemiologically linked cases across five of the six health zones for which we had obtained sequence data from samples. The size of the circles is proportional to the number of cases, and the hatch marks indicate the number of substitutions along each edge. Panel B shows a maximum clade credibility tree with a two-rate clock model in which branches indicating persistent infection were allowed to have a rate of evolution that was different than that in the rest of the tree. The tree was estimated with the use of 297 sequenced isolates with greater than 95% coverage from the current North Kivu EVD outbreak in the DRC; these isolates were differentiated according to health zone. The branch colors indicate the evolutionary rate in substitutions per genomic site per year. Black circles indicate internal nodes of the tree with a posterior probability of greater than 50%. An enlarged view of the time tree shows the first episode (sample d1) and second episode (sample d171) of EVD in the patient with relapse, as well as 61 viral genomes sampled from epidemiologically linked cases. The 95% highest posterior density of the estimated time to the most recent common ancestor (TMRCA) for the relapse clade is shown. The median TMRCA was estimated to be November 7, 2019 (95% highest posterior density interval, October 15 to November 24). The evolutionary rate between samples d1 and d171 is four times lower than that in the overall outbreak. Data are from Nextstrain (<https://nextstrain.org/community/inrb-drc/ebola-nord-kivu>) and were released on the National Center for Biotechnology Information GenBank database.

We performed a Bayesian phylodynamic analysis to reconstruct a time-resolved phylogeny with the use of all the North Kivu outbreak EBOV genomes with at least 95% coverage (Figure 24B). We determined the overall rate of evolution for the EBOV Ituri variant in the ongoing North Kivu outbreak to be 0.8×10^{-3} substitutions per genomic site per year (95% highest posterior density interval, 0.7×10^{-3} to 0.9×10^{-3}), which is consistent with the rates observed during the 2013–2016 West African epidemic.⁹ The branch leading to sample d171 had a reduced rate of 0.2×10^{-3} substitutions per genomic site per year (95% highest posterior density interval, 0.1×10^{-3} to 0.3×10^{-3}) (Figure 24B and Figure 26). This slowing of the molecular clock was consistent with persistent EBOV infection.¹⁰⁻¹³ The median estimated time to the most recent common ancestor (TMRCA) of all relapse clade genomes was November 7, 2019 (95% highest posterior density interval, October 15 to November 24), which is consistent with recurrence of symptoms in the patient on November 25 and with onward transmission shortly after. Taken together, our phylogenetic and epidemiologic data show that the patient's second EVD episode was the result of EVD relapse from his initial EBOV infection and was not due to reinfection.

To investigate the potential failure of vaccine protection at the patient's initial clinical presentation and relapse, we assayed the patient's samples for anti-EBOV glycoprotein IgG antibody titers. We were unable to detect anti-EBOV glycoprotein IgG in sample d1, but we detected high titers in a sample obtained 14 days later (in sample d14) and in a sample obtained 8 days after the onset of relapse-associated illness (sample d171) (Table 17 and Figure 27). On the basis of the half-life of Ansuvimab (approximately 24 days),¹⁴ the sample d14 results partially indicated detection of residual Ansuvimab. However, more than 99% of Ansuvimab would have been cleared when sample d171 was obtained. The higher titer in sample d171 probably resulted from recall antibody responses to the recurring infection, primary antibody responses to the recurring infection, or both, in the patient. Thus, the relapse is not explained by a failed antibody response, and some other immune deficit (acquired or primary immune deficiency) may have played a role. Given the patient's negative HIV test, we investigated the possibility of primary immune deficiencies to explain the patient's course of disease and vaccine failure. We sequenced the patient's exome, but

no variants that were known to or likely to cause primary immune deficiencies were detected (Table 20 and Figure 28).

Although the location of the mutations (G6800A/E258K and A6867G/E280G) in the viral envelope glycoprotein are distant from the Ansuvimab binding site,⁷ it is reasonable to consider whether these mutations resulted from viral escape from Ansuvimab treatment. To test for this possibility, we evaluated the ability of Ansuvimab to neutralize viruses bearing the mutant glycoproteins (Table 17 and Figure 29). The data showed nearly identical neutralization by Ansuvimab of both wild-type Ituri and the glycoprotein variant detected in our patient's viral genomes; thus, *in vitro*, the glycoprotein variation was independent of Ansuvimab selective pressures.

2.6.4. Discussion

We describe a case of acute EVD relapse that has led to human-to-human transmission. We were able to sequence EBOV genomes in samples obtained during the patient's first EVD episode (sample d1) and second EVD episode (sample d171), and we found that they differed by only two mutations. These findings indicate a relapse of the initial EBOV infection. Our time-aware phylodynamic analysis showed that between samples d1 and d171, the virus evolved at a rate that was one quarter of that in the overall outbreak; this finding indicates relapse from a persistent EBOV infection. The median TMRCA for the relapse clade of November 7, 2019, is an estimate of the earliest time point when the virus probably exited persistence and resumed a normal rate of replication. If we assume a presymptomatic incubation period of 2 to 21 days, this date is consistent with the recurrence of EVD symptoms on November 25 reported by the patient.

Although the underlying mechanism of relapse associated with EBOV persistence in convalescent patients remains unclear, three patients with documented cases of relapse (including the patient reported here) all received antibody-based therapy as part of the treatment for the initial infection. The Pamoja Tulinde Maisha (PALM ["Together Save Lives" in the Kiswahili language]) randomized, controlled trial showed the benefits of monoclonal antibody-based therapy.¹⁵ Questions remain as to whether passive immunotherapy could, in rare instances, be associated with viral relapse, as has been historically documented in patients with Argentine hemorrhagic fever treated with

convalescent plasma.¹⁶ Two previous patients with EVD relapse were repatriated persons who had severe EVD and received aggressive supportive care along with convalescent plasma and experimental therapeutic agents.^{3,17,18} Both patients recovered, and during convalescence, organ-specific inflammatory syndromes (uveitis and meningoencephalitis) developed that warranted additional treatment.^{3,4}

The major and very consequential distinction between our patient and previous patients with relapse is the extent of onward transmission. Our patient was symptomatic in the community for 8 days and visited two health care centers. Precautions were not taken to prevent the spread of infection, and there were 29 directly linked cases of EVD. This case report provides an unfortunate proof-of-principle that survivors of EVD with relapse syndromes, like patients with acute EVD, can transmit EBOV.

To investigate why the patient was not protected from infection after his vaccination in December 2018, we tested a serum sample obtained during his initial EVD episode in June 2019 for anti-EBOV glycoprotein IgG titers, but we detected none (Table 17). Given that we found no signs of immune deficiencies, this probably was an incidence of temporal or complete vaccine failure. This finding is in line with those of other studies showing that up to 10% of patients with EVD had been fully vaccinated for at least 10 days before admission to the ETU.¹⁵ In addition, serologic data from the Partnership for Research on Ebola Virus in Liberia (PREVAIL) trials involving more than 700 participants who had received rVSV-ZEBOV showed that in approximately 20% of vaccinated persons, positive Ebola IgG binding titers did not develop 1 month after vaccination.^{19,20} Combined, these findings arouse concerns about the true effectiveness of rVSV-ZEBOV, which has been estimated to be 100% in the Guinean “Ebola ça suffit!” trial²¹ and 97.5% in the preliminary report by the World Health Organization on the use of this vaccine in the DRC.²²

An alternative hypothesis for the patient’s lack of protection during relapse, despite his anamnestic response, is potential viral escape during persistence. The E280G glycoprotein mutation that developed during EBOV persistence may have allowed a replication advantage or immune escape, but our data show that the mutated glycoproteins retained sensitivity to the treatment antibody Ansuvimab. Alternatively, the mutation may simply be coincidental, and viral persistence may have arisen with infection of an immune-privileged

compartment. The other noted mutation in this patient was in a noncoding region, and little is known about the effect of an intra-genic region on gene expression in EBOV. Furthermore, our patient did not have overt evidence of chronic diseases associated with immunosuppression, and our whole-exome sequencing analysis did not reveal genomic variants that are known to or likely to cause primary immune deficiencies, although primary immunodeficiency cannot be fully ruled out.

During the North Kivu outbreak in the DRC, the provision of effective therapeutic agents for EVD and supportive care have helped more than 1000 patients to leave ETUs as survivors (who are aptly called “vainqueurs” or victors, in French).¹⁵ Despite the positive effect these countermeasures may have had on individual lives, the overall case fatality rate of approximately 66% is similar to those observed during previous outbreaks.²³ This high case fatality rate can be partially attributed to the fact that the outbreak occurred in a conflict zone, with frequent disruptions to all aspects of the outbreak response.^{24,25} This case report shows the need for continued monitoring of vaccine and therapeutic interventions and the power of having locally available genomic capabilities to support the outbreak response. Relapse of EVD appears to be a rare event ; however, relapse, like sexual transmission, should be recognized as a mechanism for onward transmission from persons with persistent infections. More data are needed to understand the mechanism and risk factors of EVD relapse in order to prevent future transmission events and protect patients as well as their families and communities.

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APPENDIX

The authors' full names and academic degrees are as follows: Placide Mbala-Kingebeni, M.D., Ph.D., Catherine Pratt, M.Sc., Mbusa Mutafali-Ruffin, M.D., Matthias G. Pauthner, Ph.D., Faustin Bile, M.D., Antoine Nkuba-Ndaye, M.D., Allison Black, Ph.D., Eddy Kinganda-Lusamaki, M.D., Martin Faye, Ph.D., Amuri Aziza, M.Sc., Moussa M. Diagne, Ph.D., Daniel Mukadi, M.D., Bailey White, B.Sc., James

Hadfield. Ph.D., Karthik Gangavarapu. B.Pharm., Nella Bisento. M.Sc., Donatien Kazadi. M.D., Bibiche Nsunda. B.Sc., Marceline Akonga. B.Sc., Olivier Tshiani. M.D., John Misasi. M.D., Aurelie Ploquin. Ph.D., Victor Epaso. M.D., Emilia Sana-Paka. M.D., Yannick Tutu Tshia N'kassar. M.D., Fabrice Mambu. M.D., Francois Edidi. M.D., Meris Matondo. M.D., Junior Bula Bula. M.D., Boubacar Diallo. M.D., Mory Keita. M.D., Marie R.D. Belizaire. M.D., Ibrahima S. Fall. Ph.D., Abdoulaye Yam. M.D., Sabue Mulangu. M.D., Anne W. Rimion. Ph.D., M.P.H., Elias Salfati. Ph.D., Ali Torkamani. Ph.D., Marc A. Suchard. M.D., Ph.D., Ian Crozier. M.D., Lisa Hensley. Ph.D., Andrew Rambaut. Ph.D., Ousmane Faye. Ph.D., Amadou Sall. Ph.D., Nancy J. Sullivan. Ph.D., Trevor Bedford. Ph.D., Kristian G. Andersen. Ph.D., Michael R. Wiley. Ph.D., Steve Ahuka-Mundeke. M.D., Ph.D., and Jean-Jacques Muyembe Tamfum. M.D., Ph.D.

The authors' affiliations are as follows: the Institut National de Recherche Biomédicale (P.M.-K., A.N.-N., E.K.-L., A.A., D.M., N.B., D.K., B.N., M.A., O.T., S.M., S.A.-M., J.-J.M.T.), the University of Kinshasa (P.M.-K., A.N.-N., F.M., F.E., M.M., J.B.B., S.A.-M., J.-J.M.T.), and Ministère de la Santé (F.B., V.E., E.S.-P., Y.T.T.N.) — all in Kinshasa, Democratic Republic of Congo; the University of Nebraska Medical Center, Omaha (C.P., B.W., M.R.W.); International Medical Corps (M.M.-R.) and the University of California, Los Angeles (A.W.R., M.A.S.), Los Angeles, and the Scripps Research Institute, La Jolla (M.G.P., K.G., E.S., A.T., K.G.A.) — all in California; the Fred Hutchinson Cancer Research Center, Seattle (A.B., J.H., T.B.); the Institut Pasteur de Dakar, Dakar, Senegal (M.F., M.M.D., O.F., A.S.); the Vaccine Research Center, National Institutes of Health, Bethesda (J.M., A.P., N.J.S.), and the Clinical Monitoring Research Program Directorate, Frederick National Laboratory for Cancer Research (I.C.), and the Integrated Research Facility at Fort Detrick, National Institutes of Health (L.H.), Frederick — all in Maryland; the World Health Organization, Geneva (B.D., M.K., M.R.D.B., I.S.F., A.Y.); and the University of Edinburgh, Edinburgh, United Kingdom (A.R.).

REFERENCES

1. Dowell SF, Mukunu R, Ksiazek TG, Khan AS, Rollin PE, Peters CJ. Transmission of Ebola hemorrhagic fever: a study of risk factors in family members, Kikwit, Democratic Republic of the Congo, 1995. *Commission de Lutte contre les Epidémies à Kikwit. J Infect Dis* 1999;179:Suppl 1: S87-S91.
2. Subissi L, Keita M, Mesfin S, et al. Ebola virus transmission caused by persistently infected survivors of the 2014-2016 outbreak in West Africa. *J Infect Dis* 2018; 218:Suppl 5:S287-S291.
3. Jacobs M, Rodger A, Bell DJ, et al. Late Ebola virus relapse causing meningoencephalitis: a case report. *Lancet* 2016;388: 498-503.
4. Varkey JB, Shantha JG, Crozier I, et al. Persistence of Ebola virus in ocular fluid during convalescence. *N Engl J Med* 2015; 372:2423-7.
5. Mbala-Kingebeni P, Aziza A, Di Paola N, et al. Medical countermeasures during the 2018 Ebola virus disease outbreak in the North Kivu and Ituri Provinces of the Democratic Republic of the Congo: a rapid genomic assessment. *Lancet Infect Dis* 2019;19:648-57.
6. Regules JA, Beigel JH, Paolino KM, et al. A recombinant vesicular stomatitis virus ebola vaccine. *N Engl J Med* 2017; 376:330-41.
7. Misasi J, Gilman MSA, Kanekiyo M, et al. Structural and molecular basis for Ebola virus neutralization by protective human antibodies. *Science* 2016;351: 1343-6.
8. Guidance for managing ethical issues in infectious disease outbreaks. Geneva: World Health Organization. 2016.
9. Holmes EC, Dudas G, Rambaut A, Andersen KG. The evolution of Ebola virus: insights from the 2013-2016 epidemic. *Nature* 2016;538:193-200.
10. Arias A, Watson SJ, Asogun D, et al. Rapid outbreak sequencing of Ebola virus in Sierra Leone identifies transmission chains linked to sporadic cases. *Virus Evol* 2016;2(1):vew016.
11. Blackley DJ, Wiley MR, Ladner JT, et al. Reduced evolutionary rate in reemerged Ebola virus transmission chains. *Sci Adv* 2016;2(4):e1600378.
12. Mate SE, Kugelman JR, Nyenswah TG, et al. Molecular evidence of sexual transmission of Ebola virus. *N Engl J Med* 2015;373:2448-54.
13. Whitmer SLM, Ladner JT, Wiley MR, et al. Active Ebola virus replication and heterogeneous evolutionary rates in EVD survivors. *Cell Rep* 2018;22:1159-68.
14. Gaudinski MR, Coates EE, Novik L, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of the therapeutic monoclonal antibody Ansumvimab targeting Ebola virus glycoprotein (VRC 608): an open-label phase 1 study. *Lancet* 2019; 393:889-98.
15. Mulangu S, Dodd LE, Davey RT Jr, et al. A randomized, controlled trial of Ebola virus disease therapeutics. *N Engl J Med* 2019;381:2293-303.
16. Maiztegui JI, Fernandez NJ, de Damielano AJ. Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome. *Lancet* 1979;2:1216-7.
17. Connor MJ Jr, Kraft C, Mehta AK, et al. Successful delivery of RRT in Ebola virus disease. *J Am Soc Nephrol* 2015;26: 31-7.
18. Kraft CS, Hewlett AL, Koepsell S, et al. The use of TKM-100802 and convalescent plasma in 2 patients with Ebola virus disease in the United States. *Clin Infect Dis* 2015;61:496-502.
19. Bolay FK, Grandits G, Lane HC, et al. PREVAIL I cluster vaccination study with rVSVΔG-ZEBOV-GP as part of a public health response in Liberia. *J Infect Dis* 2019;219:1634-41.
20. Kennedy SB, Bolay F, Kieh M, et al. Phase 2 placebo-controlled trial of two vaccines to prevent Ebola in Liberia. *N Engl J Med* 2017;377:1438-47.
21. Henaou-Restrepo AM, Camacho A, Longini IM, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). *Lancet* 2017;389:505-18.
22. Preliminary results on the efficacy of rVSV-ZEBOV-GP Ebola vaccine using the ring vaccination strategy in the control of an Ebola outbreak in the Democratic Republic of the Congo: an example of integration of research into epidemic response. World Health Organization. April 12, 2019 (<https://www.who.int/csr/resources/publications/ebola/ebola-ring-vaccination-results-12-april-2019.pdf?ua=1>).
23. Di Paola N, Sanchez-Lockhart M, Zeng X, Kuhn JH, Palacios G. Viral genomics in Ebola virus research. *Nat Rev Microbiol* 2020;18:365-78.
24. Jombart T, Jarvis CI, Mesfin S, et al. The cost of insecurity: from flare-up to control of a major Ebola virus disease hotspot during the outbreak in the Democratic Republic of the Congo, 2019. *Euro Surveill* 2020;25(2):1900735.
25. Wells CR, Pandey A, Ndeffo Mbah ML, et al. The exacerbation of Ebola outbreaks by conflict in the Democratic Republic of the Congo. *Proc Natl Acad Sci U S A* 2019;116:24366-72.

Supplementary Appendix

Ebola virus transmission initiated by systemic Ebola virus disease relapse

Methods

Ethics statement

The use of Ansuvimab during the June 2019 treatment was conducted under the Monitored Emergency Use of Unregistered and Investigational Interventions (MEURI) protocol which was reviewed by Institut National de Recherche Biomédicale internal review board (IRB). The Ministry of Health (MoH) of the Democratic Republic of the Congo (DRC) approved this study. Oral consent was obtained at the homes of patients or in the Ebola Treatment Units prior to any sample collection by a team, including staff members of the MoH.

The sequencing and analysis of de-identified human diagnostic surveillance samples analyzed in this outbreak response project were also reviewed for the applicability of human subjects protection regulations by IRBs from Scripps and UNMC and was determined to not be human subject research.

Authors contributions

PMK, CP, MMR, MGP, TB, NJS, KGA, MRW, SAM, and JJMT designed the study. PMK, CP, MMR, MGP, FB, ANN, EKL, MF, AA, MMD, DM, BW, NB, DK, BN, MA, OT, AP, VE, ESP, YTTN, FM, FE, MM, JBB, BD, MK, MRDB, ISF, AY, MS, AWR, OF, and AS gathered the data. CP, MGP, AB, EKL, JH, KG, JM, ES, AT, MAS, IC, LH, AR, NJS, TB, KGA, and MRW analyzed the data. All authors vouch for the data and the analysis. PMK, CP, MMR, MGP, FB, ANN, AB, JH, KG, JM, IC, LH, TB, KGA, and MRW wrote the paper, and all authors listed in by-line decided to publish the paper. PMK, CP, MMR, MGP, AB, TB, KGA, and MRW wrote the first draft of our manuscript.

Viral sequencing

RNA was extracted from serum samples using the Qiagen Viral RNA Mini kit. cDNA was prepared using the ThermoFisher 1st strand synthesis system, and amplicons prepared using Q5 mastermix (New England Biolabs) and EBOV-specific amplicons generated using PrimalSeq.¹ Amplicons were quantified using a Qubit fluorometric quantification device (ThermoFisher) with dsDNA broad range kit and diluted to <500 ng for input into library

preparation. Libraries were prepared using the Illumina Nextera DNA Flex kit with IDT for Illumina Unique Dual indexes. Quantified, and loaded on the Illumina iSeq 100 for 2 x 150 cycles or Illumina MiSeq for 2 x 150 cycles.

Viral sequencing analysis

Short read data were analyzed with the iVar (v1.0.1)² using reference sequence MK007330. Thereads were trimmed using the ivar trim command with a quality threshold of 20 and with option – e, to prevent the removal of reads that are not directly attached to amplicon primers following Nextera Flex library prep. Consensus sequences were called using the ivar consensus command, and a minimum coverage depth threshold of 50x, to prevent inadvertent contamination. Negativecontrols were included in each run to monitor potential contamination.

Multiple sequence alignment was performed with MAFFT³ after retaining only genomes that were >95% complete. The maximum likelihood analysis was performed using IQ-TREE using ModelFinder to select the best-fit model.⁴ The Nextstrain phylogenetics platform was used as part of the analysis.⁵ Bayesian phylogenetic analysis was performed using BEAST v1.10.5 to infer time-resolved phylogenies.⁶ We used an SDR06 nucleotide substitution model with a local clock model and a non-informative continuous time Markov chain reference prior (CTMC) on the molecular clock rate and a Skygrid coalescent prior. All the Bayesian analyses were run for 80 million Markov chain Monte Carlo steps, sampling parameters and trees every 10.000 generations.^{7,8} Tracer v1.7 was used to ensure run convergence (effective sample size > 200).⁹ The BEAST XML and log files are available at https://github.com/andersen-lab/paper_2020_drc-ebola. The final figures were created using baltic (<https://github.com/evogytis/baltic>) and Phylo (<https://biopython.org/wiki/Phylo>).

EBOV IgG ELISAs

Anti-human Ebola GP IgG ELISA data was generated using the respective Alpha Diagnostic International kits, according to manufacturer's instructions. In brief, patient serum was diluted as indicated. Final incubation with TMB substrate was carried out for 15 minutes, before stopping solution was added. ELISA Plates were read at 450nm, and optical

density at 630nm was subtracted to normalize well background. To analyze the data, blank background signal was subtracted from all data. EC₅₀ binding titers were determined using Graphpad Prism 8.

Human exome sequencing and analysis

Human genomic DNA was isolated from blood using the Qiagen DNA Blood and Tissue kit. Exome sequencing libraries were prepared using the Illumina Nextera Flex for Enrichment kit, utilizing the Illumina Exome Panel as enrichment oligos and IDT for Illumina Unique Dual indexes according to manufacturer's instructions. Sequencing libraries were analyzed on an Agilent TapeStation using the high-sensitivity DNA reagents before dilution and loading. Two exome libraries were prepared from two independent DNA extractions of the same sample, to maximize exome coverage. Both libraries were loaded on a single Illumina MiSeq run, using 2 x 300 cycle V3 chemistry to maximize the coverage depth. Close to 20 million unique reads were obtained, resulting in an average depth of 69X across all variants found. Sequence quality control was done using the FastQC software package ([https://](https://www.bioinformatics.babraham.ac.uk/projects/fastqc/)), and sequences were mapped to *hg19* using the BWA aligner.¹⁰ SNP and INDEL calling. Annotation, classification and in-depth analyses were carried out with the Genoox platform (<https://www.genoox.com/>). The analysis focused on genes causing immunodeficiency disorders. Upon analysis of likely pathogenic mutations using a built-in tool in the Genoox platform we identified a single likely pathogenic variant, c.356_357insC (p.Glu119fs), in the *SH2D1A* gene (Table 19). This variant has been reported to cause Lymphoproliferative Syndrome. OMIM # 308240.¹¹ However, Sanger sequencing confirmation revealed that this variant arose from a sequencing error, and was in fact not present in the patient's genome.

Neutralization assay

Ansvimab recognition of patient EBOV GP mutants was evaluated using a single-round infection and neutralization assay. Lentiviruses were produced bearing at their surface EBOV GP from either Ituri wildtype GP (first sequenced virus 18FHV089¹²), patient's first infection sequenced GP (d1. MAN4194) and patient's second infection sequenced GP (d171. MAN12309). Neutralization was performed as previously described.¹³ Briefly,

HEK293T cells were exposed to pseudovirus mixed with serial dilutions of Ansuvimab at concentrations from 0.0001–10 µg/mL. Luciferase activity measured as relative luminescence unit (RLU) was obtained after lysis of target cells by using a Luciferase Assay System Bright Glo (Promega) and an Envision Plate Reader (PerkinElmer). Assays were performed three times, each with samples in triplicate.

Supplementary Figures and Tables:

Blood chemistry data, as measured by the Piccolo Xpress system (Abaxis) on December 4 during the second Ebola episode. The results indicate multiple organ failure and the patient passed away the same day.

Table 18. Blood chemistry Data

Test	Result	Normal range
Glycemia	126 mg/dl	73-118
BUN	131 mg/dl	7-22
CRE	7.6 mg/dl	0.6-1.2
TBIL	5.4 mg / dl	0.2-1.6
ALB	1.5 g/dl	3.3-5.5
ALT	431 U/l	10-47
AST	1221 U/l	11-38
CK	1400 U/l	30-380
AMY	436 U/l	14-97
Na ⁺	126 mmol/l	128-145
K ⁺	4.0 mmol/l	3.6-5.1
CA	7.0 mg/dl	8.0-10.3
CRP	169 mg/l	0-7.5

Table 19. Sample metadata

Lab ID	Date sample tested	Health zone	Province	Genome coverage	Date sample sequenced
MAN4194	16-Jun-19	Mabalako	Nord-Kivu	99.58%	20-Dec-19
MAN12309	3-Dec-19	Mabalako	Nord-Kivu	96.67%	20-Dec-19
MAN12369	5-Dec-19	Mabalako	Nord-Kivu	99.65%	23-Jan-20
MAN12448	7-Dec-2019	Mabalako	Nord-Kivu	99.70	17-Dec-19
MAN12460	8-Dec-19	Mabalako	Nord-Kivu	99.99%	17-Dec-19
MAN12468	8-Dec-19	Mabalako	Nord-Kivu	98.55%	17-Dec-19
MAN12470	8-Dec-19	Mabalako	Nord-Kivu	99.97%	17-Dec-19
MAN12472	8-Dec-19	Mabalako	Nord-Kivu	99.23%	17-Dec-19
MAN12506	10-Dec-19	Mabalako	Nord-Kivu	99.22%	17-Dec-19
MAN12508	10-Dec-19	Mabalako	Nord-Kivu	98.41%	17-Dec-19
MAN12514	10-Dec-2019	Mabalako	Nord-Kivu	98.52	17-Dec-19
MAN12535	10-Dec-19	Mabalako	Nord-Kivu	98.31%	17-Dec-19
MAN12541	10-Dec-19	Mabalako	Nord-Kivu	99.87%	17-Dec-19
MAN12542	10-Dec-19	Mabalako	Nord-Kivu	99.56%	17-Dec-19
MAN12545	10-Dec-19	Mabalako	Nord-Kivu	95.52%	17-Dec-19
MAN12546	10-Dec-19	Mabalako	Nord-Kivu	99.97%	17-Dec-19
MAN12581	11-Dec-19	Mabalako	Nord-Kivu	99.97%	17-Dec-19
MAN12589	11-Dec-19	Biena	Nord-Kivu	98.91%	17-Dec-19
MAN12727	13-Dec-19	Mabalako	Nord-Kivu	99.85%	23-Jan-20
MAN12770	14-Dec-19	Mabalako	Nord-Kivu	99.87%	23-Jan-20
MAN12790	14-Dec-19	Mabalako	Nord-Kivu	99.85%	23-Jan-20
BTB39991	17-Dec-19	Butembo	Nord-Kivu	92.10%	10-Jan-20
MAN12952	17-Dec-19	Mabalako	Nord-Kivu	99.73%	21-Jan-20
MAN12990	18-Dec-19	Mabalako	Nord-Kivu	99.66%	21-Jan-20
MAN12999	18-Dec-19	Mabalako	Nord-Kivu	99.85%	27-Feb-20
MAN13030	19-Dec-19	Mabalako	Nord-Kivu	99.27%	27-Feb-20
MAN13175	21-Dec-19	Mabalako	Nord-Kivu	99.99%	2-Mar-20
MAN13221	22-Dec-19	Mabalako	Nord-Kivu	96.76%	23-Jan-20
MAN13222	22-Dec-19	Mabalako	Nord-Kivu	99.79%	21-Jan-20
MAN13238	22-Dec-19	Mabalako	Nord-Kivu	99.91%	23-Jan-20
MAN13273	23-Dec-19	Mabalako	Nord-Kivu	99.89%	11-Jan-20
MAN13347	24-Dec-19	Mabalako	Nord-Kivu	99.88%	21-Jan-20
BTB41146	25-Dec-19	Butembo	Nord-Kivu	99.91%	11-Jan-20
MAN13348	25-Dec-19	Mabalako	Nord-Kivu	99.88%	23-Jan-20
MAN13384	25-Dec-19	Mabalako	Nord-Kivu	99.91%	21-Jan-20
KAT21808	31-Dec-19	Butembo	Nord-Kivu	88.81%	11-Jan-20

Table 20. Exome variants potentially linked to primary immune deficiencies

Gene	Variation Type	Chr	Start Position	Stop Position	Ref	Alt	Transcript	AA Change	Nucleotide	Exon	Zygoty	Region	Effect	Confidence	Quality	Genotype C	Genotype I	Depth	Ref Depth	Obs Depth	Genoox Classification
CFTR	SNP	chr7	117306984	117306984	G	A	NM_000492.3	p.Arg1422Gln	c.4265G>A	27	het	Exonic	Missense	High	927.77	99	956:0:1614	135	80	55	Uncertain - Possibly Pathogenic (Low)
CIITA	SNP	chr16	10992836	10992836	T	C	NM_000246.3	p.Val138Ala	c.413T>C	5	het	Exonic	Missense	High	876.77	99	905:0:2275	172	114	58	Uncertain - Possibly Pathogenic (Low)
SH2D1A	Indel	chrX	123505210	123505210	A	AC	NM_002351.4	p.Glu119fs	c.356_357insC	4	het	Exonic	Frameshift	Low	13.9273	14		13	10	3	Likely Pathogenic
GHR	SNP	chr5	42713631	42713631	G	T	NM_000163.5		c.875+10G>T	8	het	Splice Region		Medium	137.77	99	166:0:380	27	17	10	Uncertain Significance
GHR	SNP	chr5	42718592	42718592	T	C	NM_000163.5	p.Ile328Thr	c.983T>C	10	het	Exonic	Missense	High	502.77	99	631:0:466	54	26	28	Uncertain Significance
XIAP	SNP	chrX	123025117	123025117	A	T	NM_001167.3	p.Gln336Leu	c.1007A>T	4	het	Exonic	Missense	Low	10.6282	11		10	8	2	Uncertain Significance
SDHB	SNP	chr1	17354329	17354329	G	A	NM_003000.2	p.Ser152Phe	c.455C>T	5	het	Exonic	Missense	High	902.77	99	931:0:1583	135	81	54	Uncertain - Possibly Pathogenic (Low)
C5	SNP	chr9	123737151	123737151	C	T	NM_001735.2	p.Arg1308His	c.3923G>A	30	het	Exonic	Missense	High	620.77	99	649:0:550	64	30	34	Uncertain Significance
OFD1	SNP	chrX	13774707	13774707	A	T	NM_003611.3	p.Glu411Val	c.1232A>T	13	het	Exonic	Missense	Low	23.842	21		4	2	2	Uncertain - Possibly Pathogenic (Low)

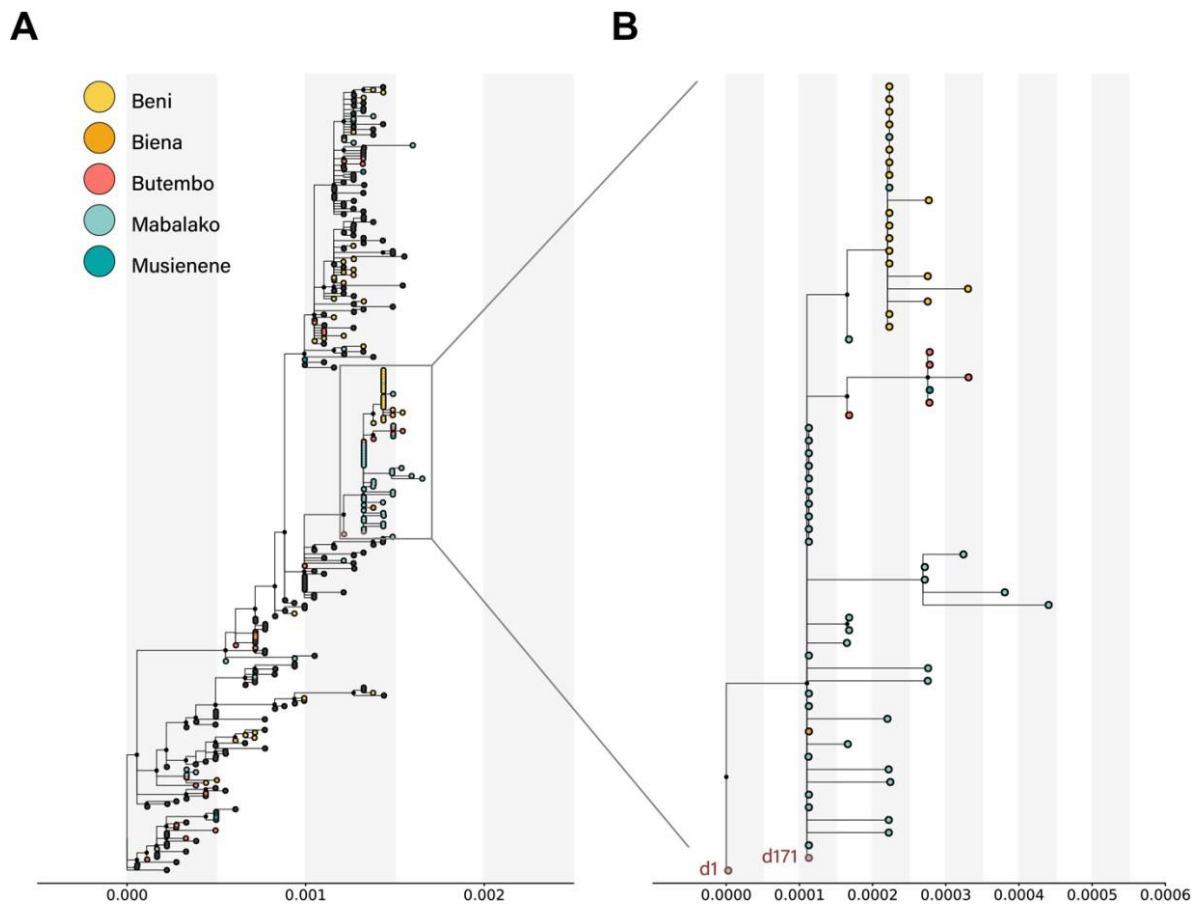


Figure 25. Maximum Likelihood tree of 297 genomes from the current Nord-Kivu EBOV outbreak in DRC

(A) Maximum Likelihood (ML) tree of sequenced isolates from the current EBOV outbreak in DRC (n=297), colored by health zones relevant to this study as indicated, (B) Zoomed in view of the ML tree showing the first (d1) and second EVD episodes (d171) of the relapse patient, as well as 61 viral genomes sampled from epidemiologically linked cases. Two mutations (T5578C - non-coding, A6867G - GP E280G) developed during the persistent infection of the relapse patient and are unique to Sample d171 and the 61 samples from the relapse cluster, showing human-to-human transmission originating from the relapse patient. The horizontal axes show the number of nucleotide substitutions per site. Data taken from <https://nextstrain.org/community/inrb-drc/ebola-nord-kivu> and released on NCBI GenBank database.

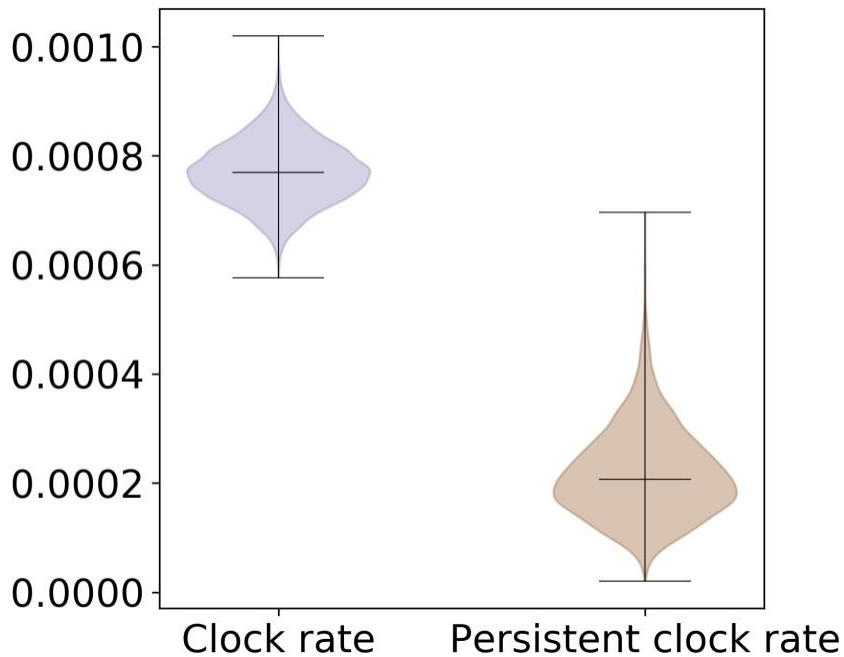


Figure 26. Comparison of evolutionary rates between persistent infection branches and the overall outbreak.

Based on preliminary Bayesian phylogenetic analysis using Beast with a relaxed clock model, we allowed three branches originating from samples d1, KAT21596 and MAN14985, which are strongly presumed to represent persistent infections, to have a different evolutionary rate from the rest of the tree, under a local clock model. This allows for the comparison of persistent infection and overall outbreak evolutionary clock rates. The median evolutionary rate for the overall outbreak was 0.00077 substitutions/site/year (clock rate ; 95% HPD : 0.00066 - 0.00088), while the median persistent evolutionary rate was approximately 4-fold lower at 0.000207 substitutions/site/year (persistent clock rate ; 95% HPD : 0.00007. 0.00038). Shown are posteriordensity distributions with median and the range indicated by black lines.

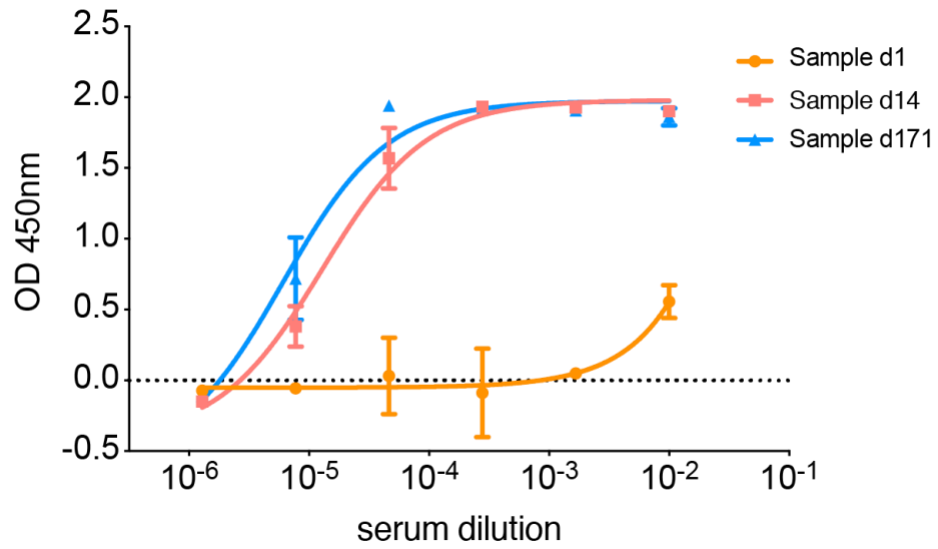


Figure 27. Anti-Ebola GP IgG ELISA data.

Anti-Ebola GP IgG ELISA results of serial serum dilutions from the first and second EVDEpisodes. Titers were calculated using Graphpad Prism 8 and are listed in [Table 17](#).

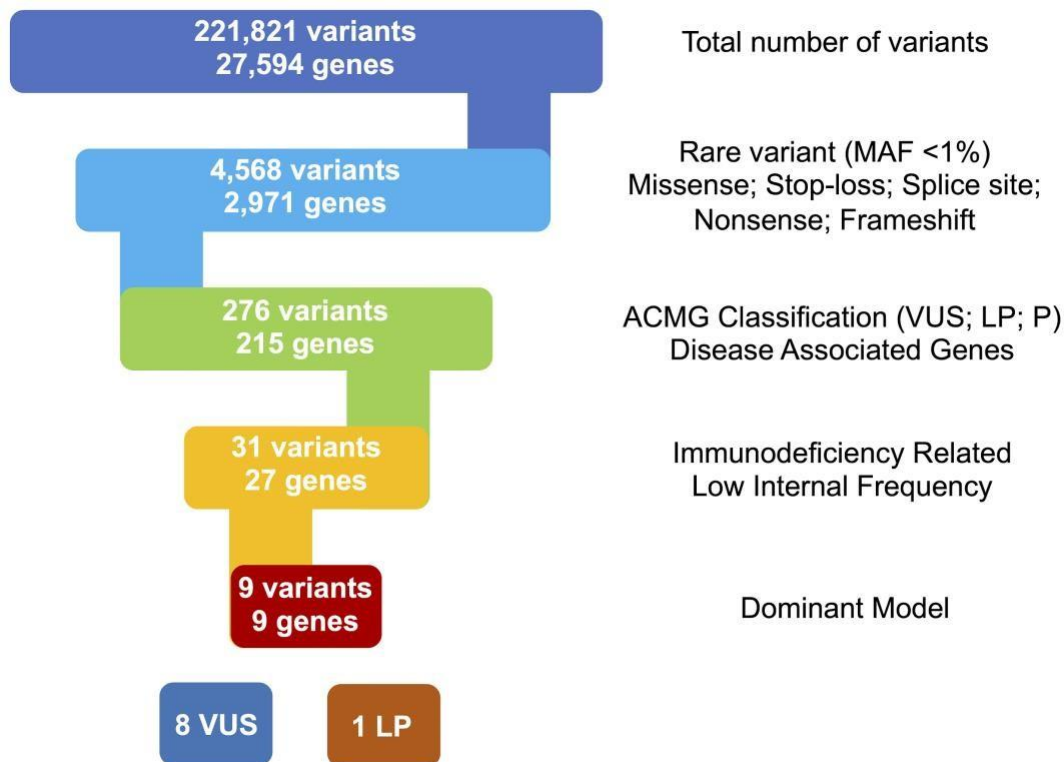


Figure 28. Exome pathogenic variant analysis summary.

Over 20 million exome reads were aligned to the human reference genome, yielding an average variant coverage of 69-fold across all 221,821 screened variants. The analysis was focused on variants that are potentially causing primary immune deficiencies, of which we found nine candidates in the filtered data set. Only one variant was found to be likely pathogenic, which we attempted to confirm with Sanger sequencing confirmation. However, the detected variant could be traced to a sequencing error, MAF : Minor Allele Frequency. ACMG : American College of Medical Genetics. VUS : Variant of Uncertain Significance. LP : Likely Pathogenic. P : Pathogenic.

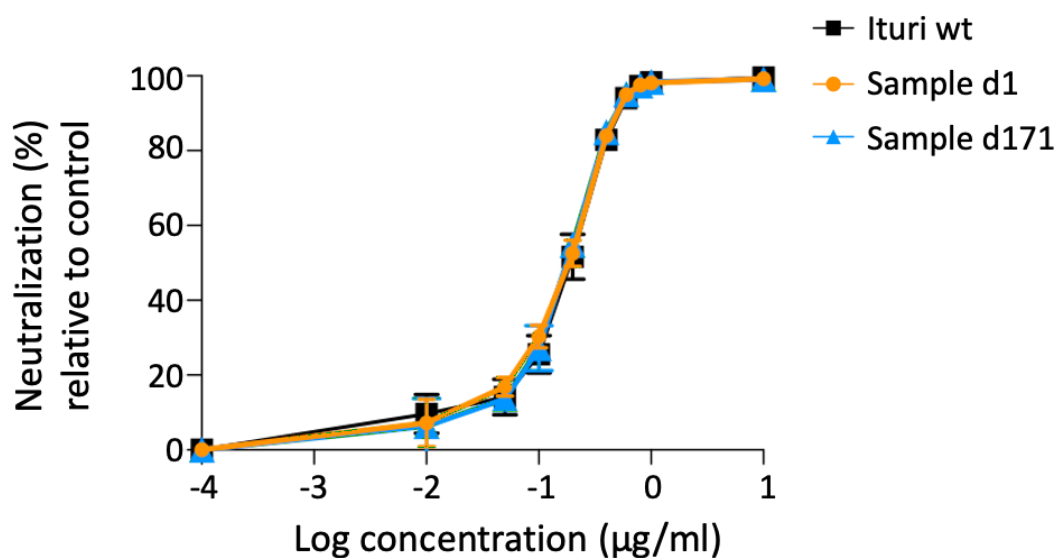


Figure 29. Ansuvimab neutralization capacity against patient's GP mutants.

Neutralization capacity of Ansuvimab was evaluated against pseudoparticles harboring either Ituri wildtype GP (18FHV089), patient's first infection GP (d1. MAN4194) and patient's second infection GP (d171. MAN12309).

References

1. Quick J. Grubaugh ND. Pullan ST. et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nat Protoc* 2017;12:1261-76.
2. Grubaugh ND. Gangavarapu K. Quick J. et al. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. *Genome Biol* 2019;20:8.
3. Katoh K. Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;30:772-80.
4. Nguyen LT. Schmidt HA. von Haeseler A. Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015;32:268-74.
5. Hadfield J. Megill C. Bell SM. et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* 2018;34:4121-3.
6. Suchard MA. Lemey P. Baele G. Ayres DL. Drummond AJ. Rambaut A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol* 2018;4:vey016.
7. Gill MS. Lemey P. Faria NR. Rambaut A. Shapiro B. Suchard MA. Improving Bayesian population dynamics inference: a coalescent-based model for multiple loci. *Mol Biol Evol* 2013;30:713-24.
8. Shapiro B. Rambaut A. Drummond AJ. Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Mol Biol Evol* 2006;23:7-9.
9. Rambaut A. Drummond AJ. Xie D. Baele G. Suchard MA. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst Biol* 2018;67:901-4.
10. Li H. Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754-60.
11. Purtle DT. Sakamoto K. Barnabei V. et al. Epstein-Barr virus-induced diseases in boys with the X-linked lymphoproliferative syndrome (XLP): update on studies of the registry. *Am J Med* 1982;73:49-56.
12. Mbala-Kingebeni P. Aziza A. Di Paola N. et al. Medical countermeasures during the 2018 Ebola virus disease outbreak in the North Kivu and Ituri Provinces of the Democratic Republic of the Congo: a rapid genomic assessment. *Lancet Infect Dis* 2019;19:648-57.
13. Corti D. Misasi J. Mulangu S. et al. Protective monotherapy against lethal Ebola virus infection by a potentially neutralizing antibody. *Science* 2016;351:1339-42.

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Mbala-Kingebeni P. Pratt C. Mutafali-Ruffin M. et al. Ebola virus transmission initiated by relapse of systemic Ebola virus disease. *N Engl J Med* 2021;384:1240-7. DOI: 10.1056/NEJMoa2024670

Thesis part 2: Characterization of antibodies against SARS-CoV-2

Chapter 1. Serology and antibody detection assays

1.1. Multiplex detection and dynamics of IgG antibodies to SARS-CoV2 and the highly pathogenic human coronaviruses SARS-CoV and MERS-CoV

Ayoub A. Thaurignac G. Morquin D. Tuailon E. Raulino R. **Nkuba A.** Lacroix A. Vidal N. Foulongne V. Le Moing V. Reynes J. Delaporte E. Peeters M. Multiplex detection and dynamics of IgG antibodies to SARS-CoV2 and the highly pathogenic human coronaviruses SARS-CoV and MERS-CoV. J Clin Virol. 2020 Aug ;129 :104521. doi : 10.1016/j.jcv.2020.104521. Epub 2020 Jun 22.

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*Corresponding author at : IRD-UMI233 TransVIHMI, 911 avenue agropolis, 349394, Montpellier, cedex 5, France.

E-mail address : ahidjo.ayouba@ird.fr (A. Ayouba).

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1.1.1. A B S T R A C T

Background: Knowledge of the COVID-19 epidemic extent and the level of herd immunity is urgently needed to help manage this pandemic.

Methods: We used a panel of 167 samples (77 pre-epidemic and 90 COVID-19 seroconverters) and SARS-CoV1, SARS-CoV2 and MERS-CoV Spike and/or Nucleopcapsid (NC) proteins to develop a high throughput multiplex screening assay to detect IgG antibodies in human plasma. Assay performances were determined by ROC curves analysis. A subset of the COVID-19+ samples (n = 36) were also tested by a commercial NC-based ELISA test and the results compared with those of the novel assay.

Results: On samples collected ≥ 14 days after symptoms onset, the accuracy of the assay is 100 % (95 % CI: 100–100) for the Spike antigen and 99.9 % (95 % CI:99.7–100) for NC. By logistic regression, we estimated that 50 % of the patients have seroconverted at 5.7 ± 1.6 ; 5.7 ± 1.8 and 7.9 ± 1.0 days after symptoms onset against Spike, NC or both antigens, respectively and all have seroconverted two weeks after symptoms onset. IgG titration in a subset of samples showed that early phase samples present lower IgG titers than those from later phase. IgG to SARS-CoV2 NC cross-reacted at 100 % with SARS-CoV1 NC. Twenty-nine of the 36 (80.5 %) samples tested were positive by the commercial ELISA while 31/36 (86.1 %) were positive by the novel assay.

Conclusions: Our assay is highly sensitive and specific for the detection of IgG antibodies to SARS-CoV2 proteins, suitable for high throughput epidemiological surveys. The novel assay is more sensitive than a commercial ELISA.

1.1.2. Introduction

In December 31th, 2019, WHO was informed on cases of pneumonia with unknown etiology in Wuhan City, Hubei Province of China and, in January 30th, the new disease was declared a public health emergency of international concern. The virus causing this severe acute respiratory syndrome (SARS) was rapidly identified as a betacoronavirus named SARS-CoV2 [1]. This new coronavirus disease, now called COVID-19, has spread globally in six months, locking down the world, infecting millions of people and killing 0.4 million of them as of June, 7th 2020. To date, there is no effective specific treatment nor prophylactic vaccine. Most countries worldwide took restrictive measures including lockdown and social distancing to flatten the epidemic curve and limit virus transmission. The novel SARS-CoV2 coronavirus induces a large spectrum of disease from asymptomatic infections to severe pneumonia and death. Thus, while expecting rapid development of effective vaccines and treatments, it is urgently needed to know the extent of the epidemic, to estimate the level of persons who have been in contact with the virus and recovered from it and the level of herd immunity [2,3].

Studies from around the world [4-10] reported on immune responses to SARS-CoV2 in the early weeks of the infection using ELISA, plaque reduction neutralization tests (PRNT), chemiluminescence or a combination of these methods. The antigens most commonly used were the spike glycoprotein S1 with the receptor binding domain [8,11], the nucleocapsid protein or both [7,9]. Assays such as PRNT and neutralization are not suited for large scale high throughput surveys as it is currently needed for SARS-CoV2 serology because they are time- and bench work-demanding, especially if two antigens are used. There is thus a need for alternative methods for screening in the context of epidemiological surveys. In earlier works on other viral infections, we have developed highly sensitive and specific microspheres bead-based tests to detect antibodies in human and wildlife samples to identify antibodies to a wide diversity of HIV/SIV and Ebola viruses [12,13]. Here, we developed an assay using the same technology to simultaneously detect IgG antibodies to the highly pathogenic human coronaviruses, SARS-CoV1, SARS-CoV2 and MERS-CoV, using two viral antigens for each of the SARS viruses. The assay presented an accuracy of 100 % and 99.9 % to detect SARS-CoV2 spike and nucleocapsid, respectively.

1.1.3. Material and methods

1.1.3.1. Human plasma

We used a panel of 167 samples (Table 21) to validate our assay. Of these, 77 were COVID-19 negative and were collected in 2015 as described earlier [13]. The remaining 90 samples were from consenting COVID-19 patients hospitalized in Montpellier University hospitals and included in the “COVIDOtheque cohort” (ClinicalTrials.gov Identifier : NCT04347850). The cohort received an institutional ethics committee approval (CPP Ile de France III, n°2020-A00935-34). They were collected between March, 26th and April, 25th, 2020 from RT-qPCR confirmed COVID-19 cases as described earlier [14].

1.1.3.2. Recombinant proteins

We used commercially available recombinant Nucleocapsid and/or Spike (S1) proteins derived from SARS-CoV1, SARS-CoV2 and MERS-CoV. The proteins were from Sinobiologicals and purchased as lyophilized powders from Interchim (Montluçon, France) and resuspended in a buffer and at concentration as per manufacturer’s instructions, aliquoted and stored until use.

1.1.3.3. Protein coupling to Luminex beads and multiplex screening for IgG antibodies to SARS-CoV1, SARS-CoV2 and MERS-CoV in plasma

We described in detail in our previous works the protocol for coupling proteins and peptides to Luminex microsphere beads [12,13]. In brief, recombinant spike proteins (1 $\mu\text{g}/1.25 \times 10^6$ beads) and nucleocapsid (2 $\mu\text{g}/1.25 \times 10^6$ beads) were covalently coupled on carboxyl functionalized fluorescent magnetic beads (Luminex Corp., Austin, TX) with the BioPlex amine coupling kit (Bio-Rad Laboratories, Marnes-la-Coquette, France) according to the manufacturer’s instructions. For each recombinant protein-coupled bead set, we used 2000 beads/ μl of assay buffer. Preliminary experiments on different plasma dilutions (1/100–1/1000) showed that the dilution 1/200 gave the best signal to noise ratio. Diluted samples were incubated with coupled beads for 16 h at 4 °C. Reactions were revealed after incubation with a biotin-labeled

anti-human IgG and streptavidin-R-phycoerythrin conjugate. Antigen-antibody reactions were read on BioPlex-200 equipment (Bio-Rad, Marnes-la-Coquette, France) and the results were expressed as median fluorescence intensity (MFI) per 100 beads. To determine IgG titers of a subset of samples against the different antigens tested, we performed a 2-fold serial dilution of these samples from 1/100 to 1/12,800 and tested them as described above. The titer was the highest value of reciprocal dilution factor given a signal above the cut-off.

1.1.3.4. Calculation of cut-off, sensitivity, specificity and accuracy

To calculate the cut-off, we used the (Mean+3xSD) formula by calculating the mean of MFI of the 77 COVID-19 negative samples for each of the recombinant proteins tested. We added to the value obtained three times the standard deviation. The result obtained was considered as the cut-off for each antigen. We also used receiver operating characteristics (ROC) curve analysis to determine the cut-off values for SARS-CoV2 antigens (because we only had convalescent samples from SARS-CoV2 patients), their sensitivity, specificity and accuracy. The ROC curve and other statistical analysis were performed with Graphpad Prism8 (San Diego, CA, USA).

1.1.3.5. Nucleocapsid based ELISA test

We used Abbott SARS-CoV-2 IgG for Alinity EIA as per manufacturer instructions to test a subset of 36 samples of the COVID-19+ samples.

1.1.4. Results

1.1.4.1. Sensitivity, specificity and accuracy to detect COVID-19 IgG in convalescent plasma

To evaluate the performance of our Luminex-based COVID-19 IgG antibody detection assay, we tested a panel of 167 samples (Table 21). The majority (71.4 %) of convalescent patients for whom the gender was specified were males and the median age was 72 years. The median duration between COVID-19 symptoms onset and sample collection was 19 days spanning from 3 to 47 days. Mean signal intensities in the COVID-19 group were 7475 ± 3576 and 7692 ± 3864 for Spike and NC, respectively. In the negative control

group, these values were 233 ± 197 and 95 ± 129 for the Spike and NC proteins, respectively.

We first calculated the cut-off values for positivity for both SARS-CoV2 Spike and Nucleocapsid recombinant proteins using the two methods described in the methods section above. For Spike recombinant protein, the cut-off values were 832 and 1030 MFI by Mean+3xSD and ROC curve analysis methods, respectively. For the nucleocapsid recombinant protein, these values were respectively 482 and 491 with Mean+3xSD and ROC curve analysis methods.

Because previous reports showed that a steady state of IgG response to a viral infection is reached at 2 weeks after exposure [5,15–17], we selected a subset of samples collected 14 days or more after onset of COVID-19 symptoms to determine the clinical performance of our assay for the detection of IgG antibodies to COVID-19. Results from that analysis (Table 22) showed that sensitivity of both recombinant proteins was 100 %. The specificity of the Spike protein was also 100% while that of Nucleocapsid was 98.7 %. The overall accuracy of both antigens taken individually was 100 % for Spike and 99.9 % for Nucleocapsid. We also combined the results of NC and Spike antigens to evaluate a sample status. By doing so, all the assay parameters (sensitivity, specificity, accuracy and the predictive values) were 100 % (Table 22).

Table 21. Characteristics of convalescent and negative control samples used in the study

Convalescents	
Sample collection date	26/03/2020-25/04/2020
Number included	90
Gender	
Male	55
Female	22
Unspecified	13
Age (years)	72
median	
Range	33- 99
Days since symptoms onset (days)	
Median	19
Range	3 - 47
Negative controls	
Collection date	December 2015
Number included	77

We next stratified the capacity of our novel assay to detect IgG directed against the spike and nucleocapsid by time after symptoms onset. We defined 3 categories: samples collected less than a week, between one week and two weeks and two weeks or more after symptoms onset. Table 23 summarizes these data and show that 50 % of patients seroconverted during the first week for both antigens, 77.7 % and 83.3 % between 1 and 2 weeks after symptoms onset on nucleocapsid and Spike, respectively. Two weeks or more after symptoms onset, 100 % of patients had seroconverted against both antigens.

Table 22. Sensitivity, specificity and accuracy of the xMAP assay to detect IgG to SARS-CoV2 antigens in 138 samples; 77 negative control samples and 61 samples from COVID-19 patients \geq days after onset of symptoms

	Spike		Nucleocapsid (NC)		Spike + and NC +	
	\geq Day14 (n= 138)	95 % CI	\geq Day14 (n= 138)	95 % CI	\geq Day14 (n= 138)	95 % CI
Sensitivity (%)	100.0	92.7-100	100.0	92.7-100	100.0	94.-100
Specificity (%)	100.0	94.2-100	98.7	92.2-100	100.0	95.2-100
Accuracy (%)	100.0	100.0-100	99.9	99.7-100	100.0	97.3-100
PPV (%)	100.0	94.1-100	98.4	91.4-99.7	100.0	94.1-100
NPV (%)	100.0	95.2-100	100.0	95.2-100	100.0	95.2-100

We used logistic regression to model IgG response to Spike, Nucleocapsid or to both antigens simultaneously as a function of time since symptoms onset. Results from this analysis (Figure 30 A, B & C), showed that 50% of the patients seroconverted at 5.9 ± 1.6 ; 5.7 ± 1.8 and 7.9 ± 1.0 days since symptoms onset against Spike, Nucleocapsid or both antigens, respectively. Virtually all the patients have seroconverted by 15–20 days after symptoms of COVID-19 were identified, generalizing and confirming the observation from raw data presented in Table 23. There were no significant differences in distribution of IgG response to Spike and nucleocapsid antigens between males and females (Figure 31).

Table 23. Sensitivity of the xMAP assay to detect IgG anti SARS-CoV2 antigens stratified by time since symptoms onset

Time since symptoms onset	N IgG anti-NC Positive n positive (%)	IgG anti-SP n positive (%)	NC + SP+ n positive (%)
≤ Day 7	10 5 (50)	5 (50)	3 (30)
Day8-Day13	19 15 (78.9)	16 (84.2)	15 (78.9)
After Day13	61 61 (100)	61 (100)	61(100)
Total	90 81 (90)	82 (91.1)	79 (87.7)

To determine IgG titers to COVID-19 antigens, we selected 6 samples from the early phase of COVID-19 symptoms (< 14 days) and 6 others from later stages (> 30 days) and tested serial dilutions of these samples until negativation. Results from these titration curves showed that (Table 24 and Figure 32), overall and as expected, IgG titers of samples from later phase were higher than those from earlier phase. This observation stands for both the Spike and the Nucleocapsid proteins. At 4 weeks or later after symptoms onset, 3/6 and 5/6 of the tested samples presented IgG titers above 12,800 against Spike and Nucleocapsid, respectively. This proportion was only 1/6 for both antigens for samples collected before 2 weeks after symptoms onset.

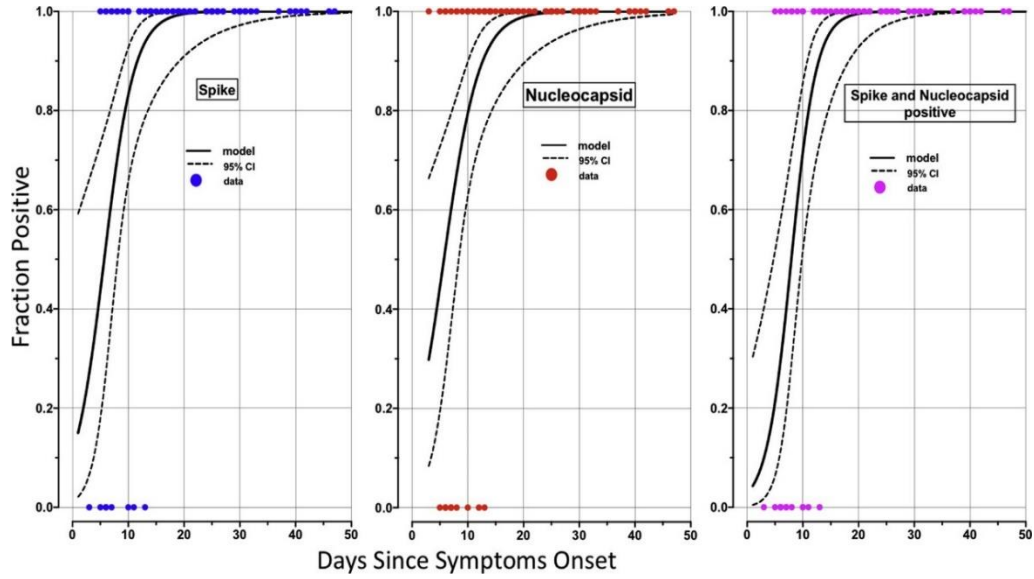


Figure 30. Timing of seroconversion during SARS-CoV2 infection.

Logistic regression was used to represent the dynamics of seroconversion in 90 COVID-19 seroconverters for Spike (left panel), Nucleocapsid (middle panel) or both (right panel). The figures show the fraction of IgG antibody positive samples as a function of time since symptoms onset. The bold curves represent the regression and dashed lines the 95 % confidence interval. All the patients have seroconverted two weeks after the onset of symptoms.

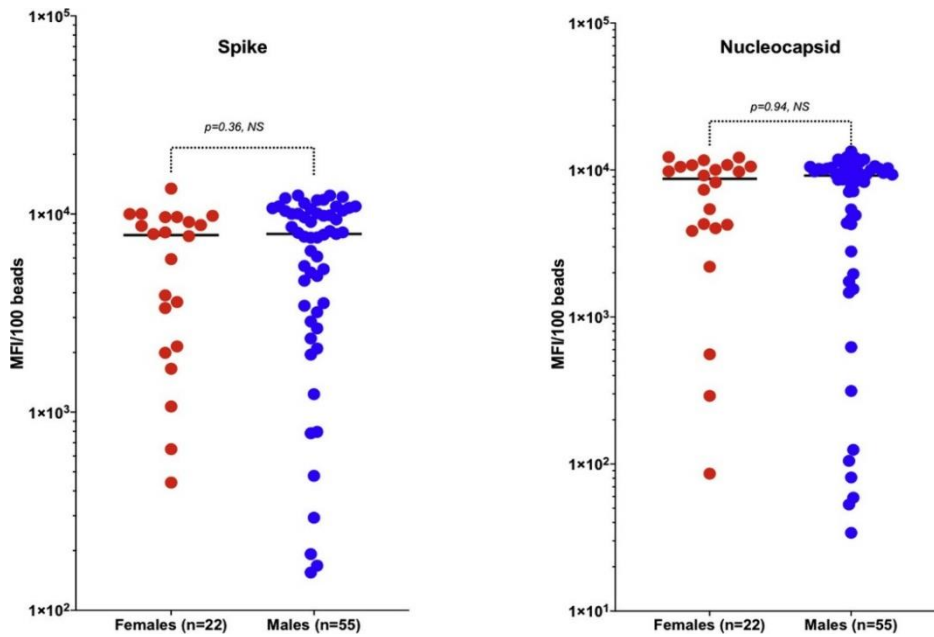


Figure 31. Comparison of IgG response to SARS-CoV2 antigens in male and female convalescent COVID 19 patients.

The figures compare IgG response to SARS-CoV2 antigens (Spike and Nucleocapsid) stratified by gender. There was no statistically significant difference between the two genders for both antigens. The groups were compared by the non-parametric Mann-Whitney U test

1.1.4.2. Cross-reactions of COVID-19 convalescent samples with SARS-CoV-1 and MERS-CoV antigens

Although very diverse, some coronavirus proteins are conserved through the different clades while others, like Spike proteins, are quite species-specific [18]. To estimate the level of antibody cross-reactions induced by SARS-CoV-2 in convalescent plasma, we also tested our positive control panel samples on the other highly pathogenic human coronaviruses, namely SARS-CoV-1 and MERS-CoV recombinant proteins. Data from Table 25 summarize the results of this comparison. Of the 61 samples of presumably fully seroconverted COVID-19+ patients (*i.e.* two weeks after symptom onset) tested on the five antigens, 100 % cross-reacted with SARS-CoV-1 Nucleocapsid protein and 45.9% also cross-reacted with SARS-CoV1 Spike protein. Notably, only 2 (3.3 %) of the 61 cross-reacted with MERS-CoV Nucleocapsid. These data are perfectly in line with the phylogenetic proximity of these viruses [19].

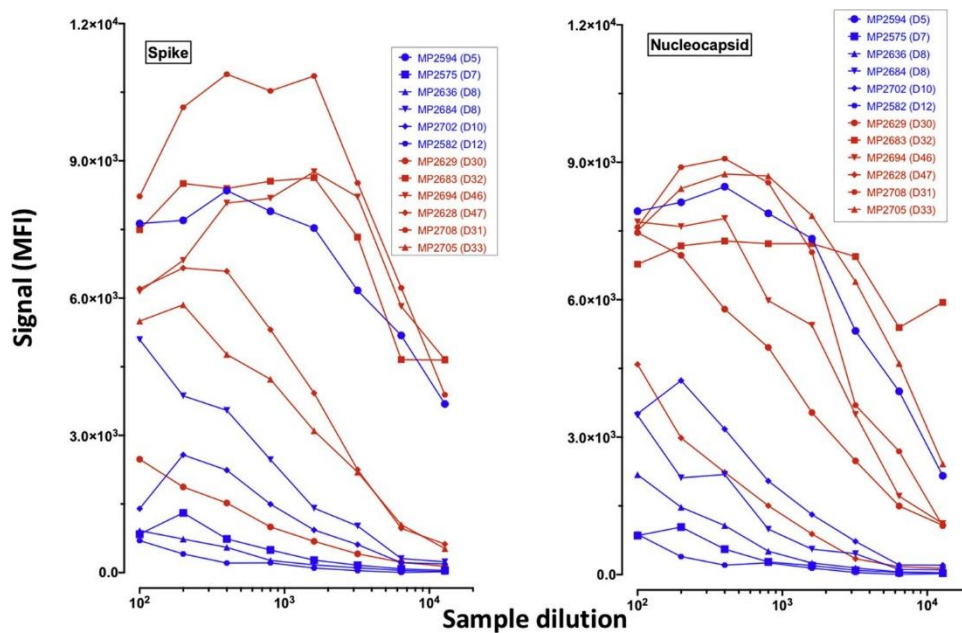


Figure 32. Titration of IgG antibodies to SARS-CoV2 Spike and Nucleocapsid recombinant proteins.

To determine the titers of IgG antibodies to SARS-CoV2 Spike and Nucleocapsid in a subset of the samples of our panel, we performed 2- fold serial dilutions on six samples from the early phase of symptoms onset and six from later phase (> 30 days). The graphs show the changes of IgG binding intensities to the Spike (left panel) and the Nucleocapsid proteins (right panel) at the different dilutions. Curves in blue are early phase samples and those in red, from later phase.

Table 24. End-point dilution titers of IgG antibodies to SARS-CoV2 Spike and Nucleocapsid recombinant proteins in a subset of early and later phase samples

Sample ID	Age	Gender	Time since symptoms onset	IgG titer Spike	IgG titer Nucleocapsid
MP2594	60	F	5	> 12,800	> 12,800
MP2575	55	M	7	200	400
MP2636	75	F	8	< 100	800
MP2684	75	F	8	3200	3200
MP2702	74	F	10	800	6400
MP2582	NA	NA	12	< 100	100
MP2629	NA	F	30	800	> 12,800
MP2708	74	M	31	> 12,800	> 12,800
MP2683	71	F	32	> 12,800	> 12,800
MP2705	73	M	33	3200	1600
MP2694	75	F	46	> 12,800	> 12,800
MP2628	68	M	47	6400	> 12,800

Table 25. Cross-reactions of 61 SARS-CoV2 convalescent samples (> 2weeks after onset of symptoms) with SARS-CoV1 and MERS-CoV antigens

	N positive/N tested	%
SARS-CoV1-NC+	61	100
SARS-CoV1-SP+	28	45.9
MERS-CoV-NC+	2	3.3

1.1.4.3. Comparison with a commercial EIA assay

To evaluate the performance of our novel assay with a commercially available EIA assay, we tested a subset of 36 samples, collected between 1 and 30 days after symptom onset, from the COVID-19+ panel. The EIA assay, United States FDA approved for emergency access, uses SARS-CoV nucleocapsid as antigen. The commercial EIA identified 29 samples positive of 36 tested (80.5 %) while our novel assay detected 31/36 (86.1 %) tested on the same NC antigen. And additional sample, negative by the commercial and was reactive on the Spike antigen. This sample was collected from a patient at day1 post symptoms onset.

1.1.5. Discussion

In most countries of the world affected by the Covid-19 pandemic, the coverage of viral detection by molecular means has been low and thus, the actual epidemic spread of the SARS-CoV2 is unknown. One possibility to fill this gap is to perform serological diagnosis and surveys. This is especially important for patients with mild to moderate illness and who do not refer to medical care, or refer later, after 2 weeks, when the probability of virus detection is low. Serological diagnosis is also an important tool to understand the extent of COVID-19 in the community and to define the level of herd immunity.

We chose the Spike and Nucleocapsid recombinant proteins as antigens because they have been shown to be highly immunogenic during coronavirus infections in humans or non-human primates [20]. Our data showed that for both antigens, the sensitivity was 100 % (Table 22). However, while the specificity of Spike antigen was also 100 %, that of the Nucleocapsid antigen was slightly lower (98.7 %) because one sample from the pre-epidemic panel reacted weakly above the cut-off threshold with that antigen. This could reflect a non-specific binding or a cross-reaction with one of the mild coronaviruses circulating in France in 2015. Overall, the accuracy of both antigens was above 99 % (Table 22). Because a fully established IgG response in a natural infection normally covers all immunogenic antigens, we also analyzed the performance of our assay by combining the two antigens we tested. As expected, this resulted in a highly sensitive and specific assay with 100% performance for all the parameters evaluated. French as well as international health authorities recommend that serological diagnostic assays should present a clinical specificity of at least 98 % and a clinical sensitivity of 90 % or more [21]. Our assay largely fulfills these criteria. When we stratified the samples by time since symptoms onset, we observed that 100 % of patients have seroconverted after two weeks (Table 23). A recent work reported that 100 % of patients (n = 125) tested for COVID-19 were IgG positive by day 17 after symptoms onset [6]. Two comprehensive reviews on different aspects of the human immune responses to coronavirus infections, including SARS-CoV2, showed that in most patients, IgG-seroconversion occurs from the second week since symptom onset onwards, with the kinetics and breadth depending on the severity or not of the disease [20,22]. However, it is too early to know if antibody response induced by SARS-CoV2 will persist over time and for how long and if they will be

protective upon re-exposure to the same or a related virus. For other human coronavirus, including SARS-CoV1 and MERS-CoV, IgG antibodies have been detected up to 2–3 years after infection [23,24].

Another major concern in the antibody response to SARS-CoV2 is the nature, breadth and titers of IgGs. Here, we found that in 8/12 samples collected ≥ 2 weeks after symptoms onset, IgG titers above 12,800 were observed. It is not known if these IgGs are neutralizing or not. Ju and colleagues [25] for instance isolated potentially neutralizing monoclonal antibodies with high titers from memory B-cells of SARS-CoV2 seroconverters.

One of the multiple advantages of the Luminex technology is the possibility of multiplexing. As previously reported [25], we observed here high-level cross-reactivity between the Nucleocapsid proteins of SARS-CoV1 and SARS-CoV2. Hence, EIA using this antigen for COVID-19 serodetection in areas where SARS-CoV1 circulated might lead to false positive results. This should be especially taken into consideration when performing epidemiological surveys. To limit this peculiarity and significantly increase the specificity of our assay, we considered a sample as positive if it was simultaneously reactive on Nucleocapsid and Spike protein. We successfully applied such an algorithm for the serology of Ebolavirus in human and wildlife samples [13,26–29]. One limitation of such a strategy is the difference in the kinetics of antibody response to these different antigens. It is very likely that surface and internal proteins will induce different kinetics of IgG responses. Hence, an algorithm combining two or more different antigens is most pertinent in the steady phase of the antibody response.

Finally, we compare our novel assay with a commercially available EIA assay. On the same viral antigen, our assay was more sensitive than the reference assay. The observation of Luminex assay being more sensitive than other EIA has already been reported by our group and others for different pathogens [13].

In summary, we have developed a highly sensitive and specific multi-target serological tool for the detection of IgG antibodies to SARS-CoV-1, SARS-CoV2 and MERS-CoV infections. The assay is at least as sensitive as a commercial EIA and is fully suited for high throughput sero-epidemiological surveys.

CRedit authorship contribution statement

Ahidjo Ayouba: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing - original draft, Writing - review & editing. Guillaume Thaurignac: Data curation, Formal analysis, Writing - review & editing. David Morquin: Resources, Writing - review & editing. Edouard Tuailon: Resources, Writing - original draft, Writing - review & editing. Raisa Raulino: Data curation, Writing - review & editing. Antoine Nkuba: Data curation, Writing - review & editing. Audrey Lacroix: Data curation, Writing - review & editing. Nicole Vidal: Data curation, Writing - review & editing. Vincent Foulongne: Resources, Writing - review & editing. Vincent Le Moing: Resources, Writing - review & editing. Jacques Reynes: Resources, Writing - review & editing. Eric Delaporte: Conceptualization, Resources, Funding acquisition, Writing - review & editing. Martine Peeters: Conceptualization, Formal analysis, Funding acquisition, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

All the authors declared no conflict of interest.

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References

- [1] L. Chen, W. Liu, Q. Zhang, K. Xu, G. Ye, W. Wu, et al., RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak, *Emerg. Microbes Infect.* (9) (2020) 313–319.
- [2] J. Goudsmit, The paramount importance of serological surveys of SARS-CoV-2 infection and immunity, *Eur. J. Epidemiol.* 35 (2020) 331–333.
- [3] F. Krammer, V. Simon, Serology assays to manage COVID-19, *Science.* 368 (2020) 1060.
- [4] A. Fontanet, L. Tondeur, Y. Madec, R. Grant, C. Besombes, N. Jolly, et al., Cluster of COVID-19 in northern France: a retrospective closed cohort study, *medRxiv.* (2020) 2020.04.18.20071134.
- [5] R. Wölfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M.A. Müller, et al., Virological assessment of hospitalized patients with COVID-2019, *Nature* 581 (2020) 465–469.
- [6] A. Bryan, G. Pepper, M.H. Wener, S.L. Fink, C. Morishima, A. Chaudhary, et al., Performance characteristics of the abbot architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho. *J Clin Microbiol.* (2020).
- [7] L. Ni, F. Ye, M.L. Cheng, Y. Feng, Y.Q. Deng, H. Zhao, et al., Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals, *Immunity* (2020).
- [8] R.A. Perera, C.K. Mok, O.T. Tsang, H. Lv, R.L. Ko, N.C. Wu, et al., Serological assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), March 2020, *Euro Surveill.* 25 (2020).
- [9] J. Qu, C. Wu, X. Li, G. Zhang, Z. Jiang, X. Li, et al., Profile of IgG and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), *Clin. Infect. Dis.* (2020).
- [10] Team C-I, Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States, *Nat. Med.* (2020).
- [11] D. Stadlbauer, F. Amanat, V. Chromikova, K. Jiang, S. Strohmeier, G.A. Arunkumar, et al., SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup, *Curr. Protoc. Microbiol.* 57 (2020) e100.
- [12] S. Ahuka-Mundeke, A. Ayouba, P. Mbala-Kingebeni, F. Liegeois, A. Esteban, O. Lunguya-Metila, et al., Novel multiplexed HIV/simian immunodeficiency virus antibody detection assay, *Emerg Infect Dis.* 17 (2011) 2277–2286.
- [13] A. Ayouba, A. Touré, C. Butel, A.K. Keita, F. Binetruy, M.S. Sow, et al., Development of a sensitive and specific serological assay based on luminex technology for detection of antibodies to Zaire ebola virus, *J. Clin. Microbiol.* 55 (2017) 165–176.
- [14] F.X. Lescure, L. Bouadma, D. Nguyen, M. Parisey, P.H. Wicky, S. Behillil, et al., Clinical and virological data of the first cases of COVID-19 in Europe: a case series, *Lancet Infect. Dis.* 20 (2020) 697–706.
- [15] K.K. To, O.T. Tsang, W.S. Leung, A.R. Tam, T.C. Wu, D.C. Lung, et al., Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study, *Lancet Infect. Dis.* 20 (2020) 565–574.
- [16] L. Guo, L. Ren, S. Yang, M. Xiao, D. Chang, F. Yang, et al., Profiling early humoral response to diagnose novel coronavirus disease (COVID-19), *Clin. Infect. Dis.* (2020).
- [17] E. Tuaille, K. Bollere, A. Pisoni, S. Debiecse, C. Renault, S. Marie, et al., Detection of SARS-CoV-2 antibodies using commercial assays and seroconversion patterns in hospitalized patients, *medRxiv.* (2020) 2020.05.04.20090027.
- [18] S. Agnihothram, R. Gopal, B.L. Yount Jr., E.F. Donaldson, V.D. Menachery, R.L. Graham, et al., Evaluation of serologic and antigenic relationships between middle eastern respiratory syndrome coronavirus and other coronaviruses to develop vaccine platforms for the rapid response to emerging coronaviruses, *J. Infect. Dis.* 209 (2014) 995–1006.
- [19] L. Joffrin, S.M. Goodman, D.A. Wilkinson, B. Ramasindrazana, E. Lagadec, Y. Gomard, et al., Bat coronavirus phylogeography in the Western Indian Ocean, *Sci. Rep.* 10 (2020) 6873.
- [20] Vabret N., Britton G.J., Gruber C., Hegde S., Kim J., Kuksin M., et al. Immunology of COVID-19: current state of the science. *Immunity.*
- [21] Haute Autorité De Santé HAS. cahier_des_charges_test_serologique_covid19', (2020) https://www.hassante.fr/upload/docs/application/pdf/202004/cahier_des_charges_test_serologique_covid19.pdf.
- [22] A.T. Huang, B. Garcia-Carreras, M.D.T. Hitchings, B. Yang, L. Katzelnick, S.M. Rattigan, et al., A systematic review of antibody mediated immunity to coronaviruses: antibody kinetics, correlates of protection, and association of antibody responses with severity of disease, *medRxiv.* (2020) 2020.04.14.20065771.
- [23] W.C. Cao, W. Liu, P.H. Zhang, F. Zhang, J.H. Richardus, Disappearance of antibodies to SARS-associated coronavirus after recovery, *N. Engl. J. Med.* 357 (2007) 1162–1163.
- [24] W. Liu, A. Fontanet, P.H. Zhang, L. Zhan, Z.T. Xin, L. Baril, et al., Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome, *J. Infect. Dis.* 193 (2006) 792–795.
- [25] B. Ju, Q. Zhang, X. Ge, R. Wang, J. Yu, S. Shan, et al., Potent human neutralizing antibodies elicited by SARS-CoV-2 infection, *bioRxiv.* (2020) 2020.03.21.990770.

- [26] A. Ayouba, S. Ahuka-Mundeke, C. Butel, P. Mbala Kingebeni, S. Loul, N. Tagg, et al., EXtensive serological survey of multiple african nonhuman primate species reveals low prevalence of immunoglobulin g antibodies to 4 ebola virus species, *J. Infect. Dis.* 220 (2019) 1599–1608.
- [27] H.M. De Nys, P.M. Kingebeni, A.K. Keita, C. Butel, G. Thaurignac, C.J. Villabona- Arenas, et al., Survey of ebola viruses in Frugivorous and insectivorous bats in Guinea, Cameroon, and the Democratic Republic of the Congo, 2015-2017, *Emerg Infect Dis.* 24 (2018) 2228–2240.
- [28] M.S.K. Diallo, M. Rabilloud, A. Ayouba, A. Touré, G. Thaurignac, A.K. Keita, et al., Prevalence of infection among asymptomatic and paucisymptomatic contact persons exposed to Ebola virus in Guinea: a retrospective, cross-sectional observational study, *Lancet Infect. Dis.* 19 (2019) 308–316.
- [29] A.K. Keita, C. Butel, G. Thaurignac, A. Diallo, T. Nioke, F. Traoré, et al., Serological evidence of ebola virus infection in Rural Guinea before the 2014 west african epidemic outbreak, *Am. J. Trop. Med. Hyg.* (99) (2018) 425–427.

1.2. Challenges in interpreting SARS-CoV-2 serological results in African countries.

Nkuba Ndaye A. Hoxha A. Madinga J. Mariën J. Peeters M. Leendertz FH. Ahuka Mundeke S. Ariën KK. Muyembe Tanfumu JJ. Mbala Kingebeni P. Vanlerberghe V. Challenges in interpreting SARS-CoV-2 serological results in African countries. *Lancet Glob Health*. 2021 May;9(5):e588-e589. doi: 10.1016/S2214-109X(21)00060-7. Epub 2021 Feb 17.

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A diagnosis of COVID-19 is based on a positive PCR test for SARS-CoV-2. Over the past year, PCR testing capacity has varied globally due to the availability of tests, and testing strategies have targeted mainly symptomatic individuals. Therefore, the spread of the virus is probably wider than the numbers reported by official surveillance systems that are based on PCR results. Serology tests detect antibodies against SARS-CoV-2, which start being measurable around 1–2 weeks after infection. They are used in seroprevalence studies to estimate the proportion of people in a population that has been infected, including asymptomatic infection. These studies are of particular importance in African countries, where reported testing and incidence are among the lowest in the world.

We did a cross-sectional serological survey of staff working in healthcare facilities in Kinshasa, the capital of the Democratic Republic of the Congo, where the first COVID-19 case was reported on March 10, 2020, in a traveller returning from France. We preselected five health zones across the city, from which we included one hospital, two health-care centres, and one COVID-19 response team. Within each facility, health-care workers and other service staff from different departments were randomly selected. Those who provided written consent to participate were enrolled in the study and a blood sample was taken, which was further analysed in the virology laboratory of the Institut National de la Recherche Biomédicale in Kinshasa. The study was approved by ethics committees in Belgium (number B3002020000144) and the Democratic Republic of the Congo (189/CNES/BN/PMMF/2020).

Between July 17 and Aug 13, 2020, 562 blood samples were collected from 562 participants and five different SARS-CoV-2 serology tests were done on each sample : two in-house

Luminex IgG based assays using recombinant nucleocapsid and spike protein 1, and three commercial assays targeting the receptor binding domain on the spike protein, all validated by the manufacturers with serum samples from Europe. These three commercial assays were the Euroimmun IgG ELISA (Euroimmun, Lübeck, Germany)—a widely used assay in Europe, with sensitivity of 94.6% and specificity of 99.8%—and Quickzen IgG and IgM (Zentech, Belgium), a lateral flow test that detects IgG and IgM antibodies on a single device with a specificity of 100% and sensitivity of 68.8% for IgM and 49.2% for IgG. The blood samples were also tested for malaria with microscopy at the same facility.

The median age of participants was 42 years ; 242 (43%) of 562 participants were male and 320 (57%) were female. 27 study participants declared having previously had a SARS-CoV-2 PCR test between March and July, of whom eight (30%) of 27 reported a positive result (1% of the total population).

202 samples (36%) were seropositive using the EUROIMMUN IgG, 171 (30%) using the Quickzen IgM and 72 (13%) using the Quickzen IgG. Only 46 (8%) of 562 samples were positive by all three tests. 89 (16%) samples were positive for malaria by microscopy. Two of eight participants who had previously tested positive by SARS-CoV-2 PCR were seronegative by all three commercial serology tests.

The Kappa coefficient, used to measure agreement between tests,² was 0.3 for the EUROIMMUN IgG assay and the Quickzen IgG assay, which is considered a minimal agreement. Because specificity for both tests is nearly 100%, but sensitivity is very different, we assessed whether all samples that were positive with the Quickzen IgG assay would be confirmed as positive with the EUROIMMUN IgG. Only 59 (82%) of 72 samples that were positive with the Quickzen IgG assay were also positive with the EUROIMMUN IgG assay.

Depending on the commercial assay used, seropositivity in healthcare staff in our study varied between 13% and 36%, which is relatively high considering the low number of symptomatic and severe cases reported in Kinshasa by the end of the study period. In other African countries, SARS-CoV-2 sero-prevalence has been estimated at 45.1% in frontline health-care workers in Nigeria (Elabscience ELISA IgG),³ 40% in women attending

antenatal clinics and people living with HIV in South Africa (Roche ECLIA IgG and IgM),⁴ 12.3% in health-care workers in Malawi (Omega ELISA spike protein and nucleocapsid protein),⁵ 5.6% in blood donors in Kenya (in-house ELISA IgG),⁶ and 3% in the general population in Ethiopia (Abbott CMIA IgG).⁷ Although these studies were done in different settings and using different serological tests, all of them tend to give a higher seroprevalence than expected on the basis of surveillance data of confirmed cases in the study period. Given our observations, part of this discrepancy could be due to the assays used.

One explanatory hypothesis for the higher than expected rate of seropositivity could be because of cross-reaction of the tests with other circulating viruses or parasites in the African subcontinent that could lower their specificity. The presence of pre-existing antibodies recognising SARS-CoV-2 in uninfected individuals due to seasonal coronaviruses was identified by Ng and colleagues,⁸ whereas Tso and colleagues⁹ showed that pre-pandemic samples from sub-Saharan Africa had higher cross-reactivity against SARS-CoV-2 than those from the USA. However, these observations need to be confirmed in larger sample sizes to assess whether prevalence of pre-existing antibodies are consistently higher in African countries. Cross-reactivity between SARS-CoV-2 and acute malaria infection was observed in a study in Benin by Yadouleton and colleagues.¹⁰ Our data do not show any correlation between participants who tested positive for malaria and those testing positive with any of the commercial SARS-CoV-2 serological tests.

Notably, SARS-CoV-2 emerged only 1 year ago and antibody response according to severity of infection and the duration of antibody persistence are not yet completely understood. However, sensitivities and specificities of serological assays can vary across populations, as shown in the early 1990s with HIV serological tests, for which lower specificity in serum samples from African individuals was observed than in samples from European individuals.¹¹

The potential lower specificity of SARS-CoV-2 commercial tests in African countries, together with the low reported prevalence of clinical cases, makes the interpretation of population surveys in this setting difficult. The seroprevalence results could be misleading and even report more false-positive cases than true-positive cases.¹²

On the basis of the low agreement between test results of our study and the available literature, we would like to warn the research community and policy makers to interpret with caution the results of seroprevalence studies done in African countries with commercial tests validated in Europe, the USA, or Asia. To establish whether these high seroprevalences are a sign of serious under-reporting of COVID-19 cases, a milder COVID-19 clinical presentation, or a cross-reaction with other circulating pathogens, we recommend in this context the use of a combination of serological tests, targeting two or more independent antigens, adjusting the cutoff values due to the overall higher background noise, or repeated serosurveys of the same population to better understand the serological profile dynamics. It will be necessary to develop standardised testing strategies, as was done in the past for serological diagnosis of HIV, and assess assays for sensitivity and specificity on reference panels that include samples from different geographical areas, including Africa.

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*Antoine Nkuba Ndaye, *Ana Hoxha, Joule Madinga, Joachim Mariën, Martine Peeters, Fabian H Leendertz, Steve Ahuka Mundeke, Kevin K Ariën, Jean-Jacques Muyembe Tanfumu, Placide Mbala Kingebeni, Veerle Vanlerberghe.*

ahoxha@itg.be

Department of Virology (ANN, SAM, J-JMT) and Department of Epidemiology (JMad, PMK), Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo; Institut de Recherche pour le Développement, University of Montpellier, Montpellier, France (ANN, MP); Virology Unit, Department of Biomedical Sciences (JMar, KKA) and Tropical Infectious Diseases Unit, Department of Public Health (AH, VV), Institute of Tropical Medicine, 2000 Antwerp, Belgium (AH); Evolutionary Ecology Group (JMar) and Department of Biomedical Sciences (KKA), University of Antwerp, Antwerp, Belgium; Epidemiology of Highly Pathogenic Microorganisms Project Group, Robert Koch Institute, Berlin, Germany (FHL); Department of Medical Microbiology, University of Kinshasa, Kinshasa, Democratic Republic of the Congo (SAM, J-JMT, PMK)

References

1. Montesinos I, Gruson D, Kabamba B, et al. Evaluation of two automated and three rapid lateral flow immunoassays for the detection of anti-SARS-CoV-2 antibodies. *J Clin Virol* 2020; 128: 104413.
2. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)* 2012;22: 276–82.
3. Olayanju O, Bamidele O, Edem F, et al. SARS-CoV-2 seropositivity in asymptomatic frontline health workers in Ibadan, Nigeria. *Am J Trop Med Hyg* 2021; 104: 91–94.
4. Hsiao M, Davies M-A, Kalk E, et al. SARS-CoV-2 seroprevalence in the Cape Town metropolitan sub-districts after the peak of infections. National Institute for Communicable Diseases. Sept 28, 2020. https://www.nicd.ac.za/wp-content/uploads/2020/09/COVID-19-Special-Public-Health-Surveillance-Bulletin_Issue-5.pdf (accessed Feb 4, 2021).
5. Chibwana MG, Jere KC, Kamng'ona R, et al. High SARS-CoV-2 seroprevalence in health care workers but relatively low numbers of deaths in urban Malawi [version 2; peer review:2 approved]. *Wellcome Open Res* 2020; 5: 199.
6. Uyoga S, Adetifa IMO, Karanja HK, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Kenyan blood donors. *Science* 2021; 371: 79–82.
7. Kempen JH, Abashawl A, Suga HK, et al. SARS-CoV-2 serosurvey in Addis Ababa, Ethiopia. *Am J Trop Med Hyg* 2020;103: 2022–23.
8. Ng KW, Faulkner N, Cornish GH, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science* 2020; 370: 1339–43.
9. Tso FY, Lidenge SJ, Pena PB, et al. High prevalence of pre-existing serological cross-reactivity against SARS-CoV-2 in sub-Saharan Africa. *Int J Infect Dis* 2021; 102: 577–83.
10. Yadouleton A, Sander AL, Moreira-Soto A, et al. Limited specificity of serologic tests for SARS-CoV-2 antibody detection, Benin. *Emerg Infect Dis* 2021; 27: 233–37.
11. Van Kerckhoven I, Vercauteren G, Piot P, van der Groen G. Comparative evaluation of 36 commercial assays for detecting antibodies to HIV. *Bull World Health Organ* 1991; 69: 753–60.
12. Peeling RW, Wedderburn CJ, Garcia PJ, et al. Serology testing in the COVID-19 pandemic response. *Lancet Infect Dis* 2020; 20: e245–49.

1.3. Evaluation of a surrogate virus neutralization test for high-throughput serosurveillance of SARS-CoV-2

Mariën J. Michiels J. Heyndrickx L. **Nkuba-Ndaye A.** Ceulemans A. Bartholomeeusen K. Madinga J. Mbala-Kingebeni P. Vanlerberghe V. Ahuka-Mundeké S. Wang LF. Ariën KK. **Evaluation of a surrogate virus neutralization test for high-throughput serosurveillance of SARS-CoV-2.** J Virol Methods. 2021 Nov;297:114228. doi: 10.1016/j.jviromet.2021.114228. Epub 2021 Jul 3. PMID: 34224754; PMCID: PMC8253660.

* Corresponding authors at: Virology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium.

E-mail addresses: joachim.marien@uantwerpen.be (J. Mariën), KArien@itg.be (K.K. Ariën).

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1.4.1. Abstract

High-throughput serological tests that can detect neutralizing antibodies against SARS-CoV-2 are desirable for serosurveillance and vaccine efficacy evaluation. Although the conventional neutralization test (cVNT) remains the gold standard to confirm the presence of neutralizing antibodies in sera, the test is too labour-intensive for massive screening programs and less reproducible as live virus and cell culture is involved. Here, we performed an independent evaluation of a commercially available surrogate virus neutralization test (sVNT, GenScript cPass™) that can be done without biosafety level 3 containment in less than 2 h. When using the cVNT and a Luminex multiplex immunoassay (MIA) as reference, the sVNT obtained a sensitivity of 94 % (CI 90–96 %) on a panel of 317 immune sera that were obtained from hospitalized and mild COVID-19 cases from Belgium and a sensitivity of 88 % (CI 81–93 %) on a panel of 184 healthcare workers from the Democratic Republic of Congo. We also found strong antibody titer correlations ($r_s > 0.8$) among the different techniques used. In conclusion, our evaluation suggests that the sVNT could be a powerful tool to monitor/detect neutralising antibodies in cohort and population studies. The technique could be especially useful for vaccine evaluation studies in sub-Saharan Africa where the basic infrastructure to perform cVNTs is lacking.

1.4.2. Introduction

One year after the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in China, more than 3.9 million fatal and 180 million diagnosed cases are recorded worldwide (Johns Hopkins University, 2021). Serosurveillance data suggests many more undiagnosed cases as national rates typically range between 5–15 % with local rates up to 50 % (Arora et al., 2020). Accurate serological data will also play a crucial role during the next phase of the pandemic. Indeed, massive vaccination campaigns are currently in progress and their efficacy needs to be monitored continuously to adjust control and prevention policies. Furthermore, while it is known that most people develop a long-lasting antibody immunity (at least 6 months after infection) (Danet et al., 2020; Duysburgh et al., 2021), the sporadic detection of re-infections in immunocompetent individuals (Selhorst et al., 2020) and the emergence of new variants that might evade the antibody response (Thomson et al., 2020) highlight the need to better understand SARS-CoV-2 antibody immunity at an individual level by directly determining the neutralizing antibody (NAb) level rather than just total binding antibodies (Babs).

While a plethora of serological tests became available months after the emergence of SARS-CoV-2, not all of them are appropriate for large-scale serosurveillance. Most high-throughput tests detect total BAbs only, such as enzyme-linked immunosorbent (ELISA), lateral flow (LFA) or multiplex (MIA) immunoassays (Marien et al., 2021). These tests can be run in basic diagnostic labs and are mainly used to confirm past infection, but are unable to directly show the presence of neutralizing antibodies in serum. The latter are typically detected with conventional virus neutralization tests (cVNT) which are labour-intensive and take 4–5 days to complete by highly trained staff in a BSL3 laboratory. Showing the direct presence and determining the level and longevity of NABs will be crucial for vaccine evaluation or serosurveillance in populations where cross-reactivity of BAbs against other related coronaviruses is likely, such as in sub-Saharan Africa (Yue et al., 2020). Furthermore, detecting NABs might be the only way to show past SARS-CoV-2 infections in particular wildlife populations for which secondary antibodies are unavailable (e.g. in populations of bat, pangolin or mink (Perera et al., 2021 ; Wacharapluesadee et al., 2021)). To overcome the difficulties of the cVNT, a surrogate viral neutralization test (sVNT) was recently developed that can be completed in 1–2 hours in a BSL2 laboratory and made

commercially available by GenScript ([Tan et al., 2020](#)). The test uses the principle of an ELISA to measure the neutralizing capacity of anti-SARS-CoV-2 antibodies by inhibiting the interactions between the receptor-binding domain (RBD) of the spike protein and ACE2 cell receptors (i.e. the main determining factor for virus neutralisation), mimicking the virus' neutralization process. Here, we performed an independent evaluation of this commercial sVNT on sera from COVID-19 cases that were screened on NAbs by cVNT and BAbs by MIA in our lab.

1.4.3. Methods

Serum samples from Belgium (n = 316) and the Democratic Republic of Congo (n = 184) were used to assess the sVNT. Our Belgian panel consisted of serum samples that were obtained at different time intervals after PCR confirmation : 163 samples were taken 1–5 weeks (recent), 45 samples 6–20 weeks (intermediate) and 108 samples 20–24 weeks (old) after PCR confirmation ([Mariën et al., 2021](#) ; [Mortgat et al., 2020](#)). All serum samples were obtained in the period between March and August 2020 in different hospitals in Belgium and were from either hospitalized COVID-19 patients (severe illness, n = 144) or healthcare workers (mild or asymptotically infected, n = 172). Of these, 60 hospitalized cases were sampled two or three times over a period of one or two weeks, while all other samples belonged to different individuals. Since we (and many others) showed that SARS-CoV-2 antibody titers decrease significantly after the initial increase and depend on disease illness ([Mariën et al., 2021](#)), this diverse panel assured that both high and low antibody titer sera were included. All Congolese samples were obtained in July-August 2020 during a cross-sectional survey of staff working in healthcare facilities in Kinshasa, the capital of the DRC. As we did not know the infection status of these participants (no PCR tests were performed), this panel included serum from people having been exposed or not to SARS-CoV-2 since the start of the pandemic in March 2020. Neutralizing antibody titers were only assessed in Congolese samples that were suggested to be positive by one of the following Bab-assays : Euroimmun Anti-SARS-CoV-2-spike IgG, Quickzen (Zentech, Belgium) IgG and IgM ([Ndaye et al., 2021](#)). All serum samples were inactivated by heating at 56 °C for 30 min.

The surrogate virus neutralisation test (sVNT) (GenScript cPass™, USA, L00847) was performed according to the manufacturer's instructions ([Tan et al., 2020](#)). Samples, positive

and negative controls were diluted 1:10 with sample dilution buffer. The dilutions were mixed with horseradish peroxidase conjugated recombinant SARS-CoV-2 RBD solution and incubated for 30 min at 37°C. The mixtures were subsequently incubated for 15 min at 37°C in a capture plate that was pre-coated with hACE2 protein. After a washing step, tetrame- thylbenzidine (TMB) solution was added and the plate was incubated in the dark at room temperature for 15 min. Stop solution was added to quench the reaction and the absorbance was immediately read at 450 nm on a ELISA microplate reader. The percentage inhibition was calculate as $1 - \left(\frac{OD \text{ value of sample}}{OD \text{ value of Negative control}} \right) \times 100\%$. Sensitivity was calculated at a low (at 20 %) and high (at 30 %) inhibition cut-offs, which corresponds to a specificity of 98 and 100 % respectively (Tanet et al., 2020).

Samples were also screened by an in-house cVNT and Luminex MIA as reference, which are described in detail in Marien et al. (2021). Briefly, for the cVNT, serial dilutions of serum (1/50-1/1600) were incubated with 3xTCID₁₀₀ of a primary isolate of SARS-CoV-2 during 1 h. This solution was added to Vero cells (18.000cells/well) in a 96 well plate and incubated for 5 days (37°C / 7 % CO₂). The cytopathic effect (CPE) caused by viral replication was scored microscopically and counted in each individual well for 8 experimental replicates per sam- ple. The Reed-Muench method was used to calculate the neutralising antibody titre that reduced the number of infected wells by 50% (cVNT₅₀) or 90% (cVNT₉₀) (Reed and Muench, 1938). Samples were still considered to be positive if more than 10 % reactivity was observed at a 1/50 serum dilution. For the Luminex MIA, recombinant receptor binding domain (RBD) and Nucleocapsid protein (NCP) (BIOCONNECT, The Netherlands) were coupled to 1.25 10⁶ paramagnetic MAGPLEX COOH-microspheres from Luminex Corporation (Texas, USA). After incubation of beads and diluted sera (1/300), a biotin-labelled anti-human IgG (1:125) and streptavidin-R-phycoerythrin (1:1000) conjugate was added. Beads were read using a Luminex® Bio-Plex 100/200 analyzer.

Samples were considered to be positive if the fluorescent signal >2X standard deviation mean of negative controls (*n* 96) for both anti- gens, which corresponds to a specificity of 99% Marien et al. (2021). Results were expressed as signal-to-noise ratios. Only 198 samples were screened on IgG BAbs using the Luminex MIA.

1.4.4. Results

From the 316 samples obtained from Belgian cases that tested PCR positive, 17 samples were seronegative for both VNTs and excluded from the sensitivity panel. While 12 of these samples were obtained <14 days after the PCR result (probably just before seroconversion started), five samples were taken 3–5 months after the PCR result. These five individuals might have been included in the panel on the basis of a false-positive PCR test or because antibodies had waned before serum sampling. Six samples that were negative in the cVNT₅₀, but positive in the Luminex MIA ($n = 3$) or the sVNT₂₀ ($n = 3$), remained in the panel. Based on this final panel, we found that the sensitivity of the sVNT₂₀ was only slightly lower than the sensitivity of the cVNT₅₀ ($\Delta 4\%$) or the Luminex MIA ($\Delta 3\%$) (Table 26). As expected, the sensitivity decreased at high specificity targets for the sVNT₃₀ ($\Delta 9\%$) and the cVNT₉₀ ($\Delta 29\%$). The group of samples ($n = 16$) that were negative on the sVNT₂₀ but positive on the cVNT₅₀ mainly consisted of sera obtained from patients <14 days after the PCR result ($n = 4$) or five months after infections ($n = 10$). For most of these samples, less than 50% (but more than 10%) inhibition was observed at a 1/50 dilution in the cVNT₅₀.

From the 184 samples obtained from Congolese participants with a positive commercial serology test, 55 tested negatives on both the cVNT and sVNT and can be considered true negatives. One sample tested negative on the cVNT and positive on the sVNT (45% inhibition), which could be a potential false-positive result on the sVNT. If the sample is indeed false-positive (we cannot rule-out the possibility of a false-negative on the cVNT), the specificity of the sVNT (relative to the cVNT) would be 98.2% (CI 90.4–99.9) based on the Congolese panel for both the 20% and 30% cut-offs. Similar as for the Belgian samples, we found that the sensitivity of the sVNT₂₀ was lower ($\Delta 11\%$) than the sensitivity of the cVNT₅₀ (Table 26).

To test if we can use the sVNT as a high-throughput alternative for the more labor-intensive cVNT, we calculated correlations (r_s) between the antibody titer proxies using the nonparametric Spearman rank test (R.3.6.1. statistical software). We found strong correlations ($r_s = 0.85$, $p > 0.0001$) between the inhibition percentage of the sVNT and

the dilution factors of the cVNT₅₀ and cVNT₉₀ (Figure 33). A strong correlation ($r_s = 0.83$, $p > 0.0001$) was also observed between the inhibition percentage of the sVNT and signal-to-noise ratios of the RBD on the Luminex MIA, but not for the NCP ($r_s = 0.44$, $p > 0.0001$) (Figure 33). The latter result is explained by the fact that the sVNT specifically detects antibodies that neutralize the RBD-ACE2 interaction.

Table 26. Sensitivity of the different serological tests and cut-offs used based on the final serum panels

Values between brackets represent 95 % confidence intervals on the estimations.

Country	Test	cVNT ₅₀	cVNT ₉₀	sVNT _{20%}	sVNT _{30%}	Luminex (Belgium) Euroimmune (DRC)
Belgium	Negative(n)	6	91	18	43	7
	Positive(n)	293	208	281	256	193
	Sensitivity	98 (95.7–99.3)	69.6 (64.0–74.7)	94.0 (90.7–96.4)	85.6 (81.1–89.4)	96.5 (92.9–98.6)
DRC	Negative(n)	1	64	15	31	10
	Positive(n)	128	65	114	98	119*
	Sensitivity	99.2 (95.7–99.9)	50.4 (41.5–59.3)	88.4 (81.5–93.4)	74 (65.4–81.8)	92.3 (86.2–96.2)

* All samples with ratio ≥ 0.8 , including borderline samples ($n = 18$).

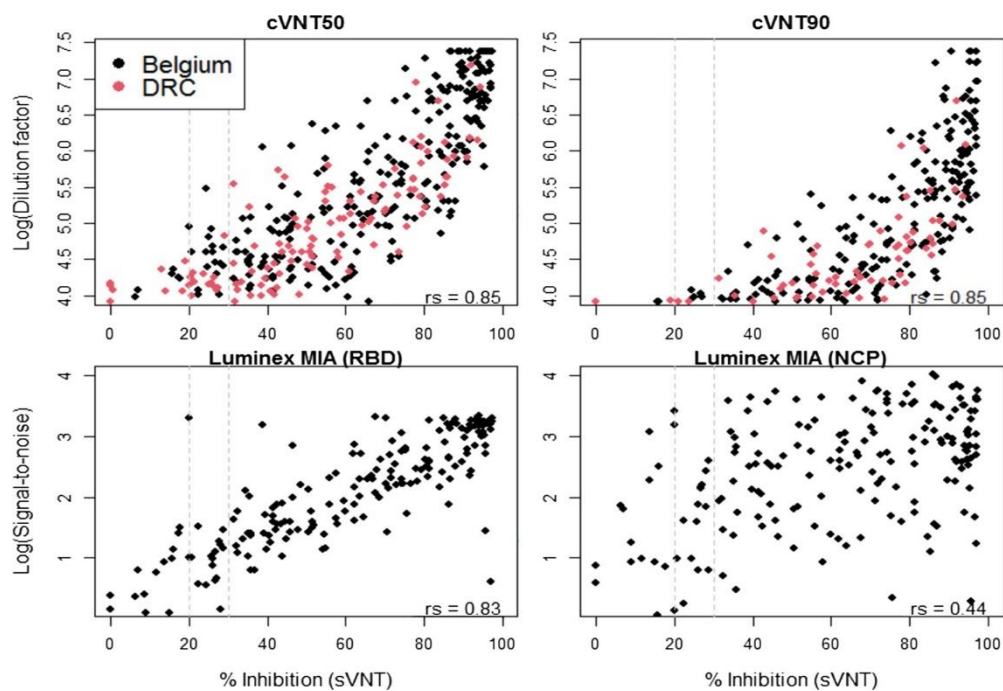


Figure 33. Correlations between the percentage of inhibition.

Measured by the surrogate viral neutralisation test (sVNT) and the log (dilution factors or signal-to-noise ratio) for the conventional viral neutralization test (cVNT) or the Luminex multiplex immunological assay (MIA) as calculated by the nonparametric Spearman correlation test (r_s). Seropositivity cut-off levels for the sVNT are indicated by the dashed grey lines at 20 or 30 % inhibition. Negative samples on the cVNT or MIA were not included in these figures.

1.4.5. Discussion

We found overall high concordance between the sVNT, the cVNT and the Luminex MIA in terms of sensitivity and antibody titer correlations. The sensitivity estimations of the sVNT₂₀ in our study are in line with [Bond et al. \(2020\)](#) who evaluated the sVNT₂₀ on serum from COVID-19 cases obtained 2–6 weeks after diagnosis in Australia. In contrast, [Meyer et al. \(2020\)](#) found a significantly lower sensitivity (83 %) on serum from COVID-19 cases >14 days after symptom onset in the Netherlands. The latter also reports a significant difference in sensitivity between the two centres where their study was conducted, potentially explained by the underrepresentation of samples taken at later time points at one of the sites. This highlights again that the timing of serum collection relative to onset of disease can affect the performance characteristics for COVID-19 serological assays.

One limitation of our study is that we cannot assess the assay performance against variants of concern (VOC, B.1.1.7, B.1.351, P.1, B.1.617.2), because we lack sequence data and strain typing on the patients from which the sera were collected. However, our sample collection precedes the global emergence of these VOCs and thus therefore is probably not a confounding factor in our analysis. The global rise of VOCs that escape neutralization by RBD-targeting antibodies might indeed affect the ability of the sVNT to assess neutralization titers against these VOCs ([Cele et al., 2021](#)). While we expect that the current assay will detect anti-RBD antibodies directed against most of the VOCs (given that cross-reactivity against SARS-CoV-1 was also noted), we warn that slight modifications to include key VOC mutations in the recombinant RBD might be needed to optimally detect neutralising capacity against these VOCs in the sVNT assay (Genscript recently released alternative sVNT assays with the B.1.1.7 and B.1.351 specific RBD sequence). Another limitation of the study is that we only included a small panel of African samples (*n* 55) to assess the specificity of the test and we cannot rule-out cross-reactivity at low titers on the cVNT, which we used as reference. However, there is overall agreement that the specificity of the sVNT is acceptably high (94–99 % at sVNT₂₀ and 99–100 % at sVNT₃₀) when evaluated on a panel containing challenging samples, including other coronaviruses (except SARS-CoV-2) or other acute infections ([Perera et al., 2021](#); [Tan et al., 2020](#); [Bond et al., 2020](#); [Meyer et al., 2020](#)).

In conclusion, our results suggest that the commercial sVNT could be a powerful tool to determine neutralising antibodies in cohort and population studies, although other high-throughput assays (such as a Luminex MIA) might outperform the sVNT in terms of individual diagnosis for evidence of infection. Another advantage of this commercial sVNT is that it allows standardization between clinical laboratories without the need to use live biological materials or biosafety containment. Together with the international unit (IU) recently established by WHO and National Institute of Biological Standards and Controls (NIBSC) ([Mattiuzzo et al., 2020](#)), this platform could be particularly useful for vaccine evaluation in sub-Saharan Africa, where diagnostic labs lack the infrastructure to run cVNTs. During serosurveillance studies, the test can also be run as an independent test to exclude cross-reactivity after the initial screening with a Luminex MIA assay. Furthermore, given that the test is both species and isotype independent, it could be used as a primary screening assay to detect reversed spillover or spillback events of SARS-CoV-2 from infected humans to wildlife populations or to find the natural reservoir of closely related sarbeco- viruses in bats or other animal populations ([Colombo et al., 2021](#); [Perera et al., 2021](#); [Wacharapluesadee et al., 2021](#)).

1.4.6. Ethics statement

Ethical approval to sample from Belgian COVID-19 cases was given by the institutional review boards of the University of Ghent, the University Hospital Antwerp and Jessa Hospital Hasselt. Ethical clearance to use samples for the evaluation of the test was given by the Institutional Review board of the Institute of Tropical Medicine Antwerp. For the Congolese samples, ethical approval was given by Institutional Review board of the Institute of Tropical Medicine Antwerp and the National Reference Laboratory INRB in Kinshasa (DRC). All participants provided informed consent to participate. We declare that the planning conduct and reporting of the study was in line with the Declaration of Helsinki, as revised in 2013.

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Author statement

Conceived the study: JMa, L-FW and KA. Wrote the paper: JMa and KA. Performed the lab experiments: JMa, JM_i, AN, LH, AC. Performed the statistical analyses: JMa. Supervised data collection and laboratory work: KB, PM, JMa_d, VvL, SA, L-FW and KA. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding or last author on reasonable request.

Declaration of Competing Interest

L-FW is a co-inventor on a patent application for the sVNT technology and a commercial kit, cPassTM, is being marketed by GenScript Biotech. Other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [Arora, R.K., Joseph, A., Van, Wyk J., Rocco, S., Atmaja, A., May, E., et al., 2020. SeroTracker: a global SARS-CoV-2 seroprevalence dashboard. *Lancet Infect. Dis.* \(January\), 19–21.](#)
- [Bond, K., Nicholson, S., Lim, S.M., Karapanagiotidis, T., Williams, E., Johnson, D., et al., 2020. Evaluation of serological tests for SARS-CoV-2: implications for serology testing in a low-prevalence setting. *J. Infect. Dis.* 222 \(8\), 1280–1288.](#)
- [Cele, S., Gazy, I., Jackson, L., Hwa, S.H., Tegally, H., Lustig, G., et al., 2021. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. *Nature* 593 \(7857\), 142–146.](#)
- Colombo, V., Sluydts, V., Mariën, J., Vanden Broecke, B., Van Houtte, N., Leirs, W., Jacobs, L., Iserbyt, A., Hubert, M., Heyndrickx, L., Goris, H., Delputte, P., De Roeck, N., Elst, J., Ariën, K., Leirs, H., Gryseels, S., 2021. SARS-CoV-2 surveillance in Norway rats (*Rattus norvegicus*) from Antwerp sewer system, Belgium. *Transboundary Emerging Dis.* <https://doi.org/10.1111/tbed.14219>. In press.
- [Dan, J.M., Mateus, J., Kato, Y., Hastie, K.M., Faliti, C.E., Ramirez, S.I., et al., 2020. Immunological memory to SARS-CoV-2 assessed for greater than six months after infection. *bioRxiv.* 4063 \(January\), 1–23.](#)
- Duysburgh, E., Mortgat, L., Barbezange, C., Dierick, K., Fischer, N., Heyndrickx, L., et al., 2021. Persistence of IgG response to SARS-CoV-2. *Lancet Infect Dis* [Internet] 21 (2), 163–164. [https://doi.org/10.1016/S1473-3099\(20\)30943-9](https://doi.org/10.1016/S1473-3099(20)30943-9). Available from:
- [Mariën, J., Ceulemans, A., Michiels, J., Heyndrickx, L., Kerkhof, K., Foque, N., et al., 2021. Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay. *J. Virol. Methods* 288.](#)
- Mattiuzzo, G., Bentley, E.M., Hassall, M., Routley, S., 2020. Establishment of the WHO international standard and reference panel for anti-SARS-CoV-2 antibody. *World Heal Organ* [Internet] (December), 9–10. Available from: <https://www.who.int/publications/m/item/WHO-BS-2020.2403>.
- [Meyer, B., Reimerink, J., Torriani, G., Brouwer, F., Godeke, G.J., Yerly, S., et al., 2020. Validation and clinical evaluation of a SARS-CoV-2 surrogate virus neutralisation test \(sVNT\). *Emerg. Microbes Infect.* 9 \(1\), 2394–2403.](#)
- Mortgat, L., Barbezange, C., Fischer, N., Heyndrickx, L., Hutse, V., Thomas, I., et al., 2020. SARS-CoV-2 prevalence and seroprevalence among healthcare workers in Belgian hospitals: baseline results of a prospective cohort study authors. *medRxiv.* <https://doi.org/10.1101/2020.10.03.20204545> [Internet]. 2020.10.03.20204545. Available from:
- [Ndaye, A.N., Hoxha, A., Madinga, J., Mariën, J., Peeters, M., Leendertz, F.H., et al., 2021. Challenges in interpreting SARS-CoV-2 serological results in African countries. *Lancet Glob Heal.* \(21\), 19–20.](#)
- [Perera, R.A.P.M., Ko, R., OTT, Y., Hui, D.S.C., Kwan, M.Y.M., Brackman, C.J., et al., 2021. Evaluation of a SARS-CoV-2 surrogate virus neutralization test for detection of antibody in human, canine, cat, and Hamster sera. *J. Clin. Microbiol.* 59 \(2\), 1–6.](#)
- [Reed, L., Muench, H.A., 1938. A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.* 27, 493–497.](#)
- [Selhorst, P., Van, Ierssel S., Michiels, J., Mariën, J., Bartholomeeusen, K., Dirinck, E., et al., 2020. Symptomatic SARS-CoV-2 re-infection of a health care worker in a Belgian nosocomial outbreak despite primary neutralizing antibody response. *Clin. Infect. Dis.* 1–18.](#)
- Tan, C.W., Chia, W.N., Qin, X., Liu, P., Chen, M.I.C., Tiu, C., et al., 2020. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. *Nat. Biotechnol.* 38 (9), 1073–1078. <https://doi.org/10.1038/s41587-020-0631-z> [Internet]. Available from:
- [Thomson, E.C., Rosen, L.E., Shepherd, J.G., Spreafico, R., Filipe, S., Wojcechowskyj, J.A., et al., 2020. The Circulating SARS-CoV-2 Spike Variant N439K Maintains Fitness While Evading Antibody-mediated Immunity.](#)
- Wacharapluesadee, S., Tan, C.W., Maneerorn, P., Duengkae, P., Zhu, F., Joyjinda, Y., et al., 2021. Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia. *Nat. Commun.* 12 (1), 972 [Internet]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/33563978>.
- [Yue, F., Lidenge, S.J., Pen a, P.B., Clegg, A.A., Wood, C., 2020. High prevalence of pre-existing serological cross-reactivity against severe acute respiratory syndrome coronavirus-2 \(SARS-CoV-2\) in sub-Saharan Africa. *Int. J. Infect. Dis.* 102 \(January\), 577–583.](#)

Chapter 2. SARS-COV-2 seroprevalence in DRC

2.1. High prevalence of anti-SARS-CoV-2 antibodies after the first wave of COVID-19 in Kinshasa. Democratic Republic of the Congo: results of a cross-sectional household-based survey

Nkuba AN. Makiala SM. Guichet E. Tshiminyi PM. Bazitama YM. Yambayamba MK. Kazenza BM. Kabeya TM. Matungulu EB. Baketana LK. Mitongo NM. Thaurignac G. Leendertz FH. Vanlerberghe V. Pelloquin R. Etard JF. Maman D. Mbala PK. Ayouba A. Peeters M. Muyembe JT. Delaporte E. Ahuka SM. **High Prevalence of Anti-Severe Acute Respiratory Syndrome Coronavirus 2 (Anti-SARS-CoV-2) Antibodies After the First Wave of Coronavirus Disease 2019 (COVID-19) in Kinshasa. Democratic Republic of the Congo : Results of a Cross-sectional Household-Based Survey.** Clin Infect Dis. 2022 Mar 9 ;74(5) :882-890. Doi : 10.1093/cid/ciab515. PMID : 34089598 ; PMCID : PMC8244674.

Received 25 April 2021; editorial decision 27 May 2021; published online 5 June 2021. Correspondence: E. Delaporte, Institut de Recherche pour le Développement, Université de

Montpellier, 911 Avenue Agropolis, 34394 Montpellier Cedex 5, France (eric.delaporte@ird.fr).

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Background. In October 2020, after the first wave of coronavirus disease 2019 (COVID-19), only 8290 confirmed cases were reported in Kinshasa, Democratic Republic of the Congo, but the real prevalence remains unknown. To guide public health policies, we aimed to describe the prevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin G (IgG) antibodies in the general population in Kinshasa.

Methods. We conducted a cross-sectional, household-based serosurvey between 22 October 2020 and 8 November 2020. Participants were interviewed at home and tested for antibodies against SARS-CoV-2 spike and nucleocapsid proteins in a Luminex-based assay. A positive serology was defined as a sample that reacted with both SARS-CoV-2 proteins (100% sensitivity, 99.7% specificity). The overall weighted, age-standardized prevalence was estimated and the infection-to-case ratio was calculated to determine the proportion of undiagnosed SARS-CoV-2 infections.

Results. A total of 1233 participants from 292 households were included (mean age, 32.4 years; 764 [61.2%] women). The overall weighted, age-standardized SARS-CoV-2 seroprevalence was 16.6% (95% CI: 14.0–19.5%). The estimated infection-to-case ratio was 292:1. Prevalence was higher among participants ≥ 40 years than among those < 18 years (21.2% vs 14.9%, respectively; $P < .05$). It was also higher in participants who reported hospitalization than among those who did not (29.8% vs 16.0%, respectively; $P < .05$). However, differences were not significant in the multivariate model ($P = .1$).

Conclusions. The prevalence of SARS-CoV-2 is much higher than the number of COVID-19 cases reported. These results justify the organization of a sequential series of serosurveys by public health authorities to adapt response measures to the dynamics of the pandemic.

Keywords. SARS-CoV-2; serological survey; general population; DRC; Africa.

2.2.1. Introduction

Coronavirus disease 2019 (COVID-19) is a highly contagious viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The infection was first identified in December 2019 in China, but has spread extremely fast worldwide, including in Africa (Juma *et al.*, 2020). In the Democratic Republic of the Congo (DRC), the first confirmed COVID-19 case was reported on 10 March 2020, in the capital city of Kinshasa, in a Congolese traveler who lived in Europe and had returned to the DRC. After the first cases, the Congolese government rapidly declared a state of emergency and set up a national multisectoral national committee to design strategies to address the pandemic (Juma *et al.*, 2020). Among the public health measures taken to control the spread of the virus, the national lockdown was first imposed in Kinshasa and then across the entire country, flights from COVID-19–infected countries were suspended, schools and universities were closed, gatherings of more than 20 people were banned, and there was an obligation to wear masks in public areas (Whembolua *et al.*, 2020).

On 19 October 2020, after the first epidemic wave (March to July), the DRC reported 11 078 confirmed COVID-19 cases, with 303 deaths. The capital city of Kinshasa represented 74% (8290) of all notified cases across the country (United Nation High Commissioner for Refugees, 2020). COVID-19 reported cases in the DRC were much lower than predicted by many researchers. For example, Walker *et al.* (Patrick G. T. Walker *et al.*, 2020) suggested that 70 million Africans could be infected by SARS-CoV-2, with more than 3 million deaths. Furthermore, Wells *et al.* (Chad R. Wells *et al.*, 2020) estimated that there would be 76 213 155 infections and 319 441 deaths in the absence of physical distancing and any public health measures in the DRC.

Several factors may explain this glaring difference between the prediction and the number of COVID-19 reported cases. First, there was limited capacity to test for SARS-CoV-2, especially in the early stage of the epidemic, but gradually the daily capacity of SARS-CoV-2 polymerase chain reaction (PCR) testing in Kinshasa increased and reached 1000, which is still insufficient. However, there was no saturation of hospital reception capacities or unexplained high mortality during the same period, even if mortality may have been underestimated. Some of the potential factors that could explain this difference are the age

pyramid, with a younger population potentially resulting in a greater number of asymptomatic cases; pre-existing immunity due to possible cross-reaction with other tropical infectious diseases or other coronaviruses; environmental factors; and early implementation of measures to control the disease (Maeda *et al.*, 2021; Nachega *et al.*, 2020).

In the African context, and following the recommendations of the World Health Organization (WHO, 2020d), population-based sero-surveillance is important to complete data on the reported cases of SARS-CoV-2 infection in order to assess the real extent of the epidemic and to enable decision makers to adjust public health response measures. Several serological surveys have been carried out in Africa and have shown a high variability of seroprevalence of SARS-CoV-2 across countries, but most were performed in specific population groups, such as blood donors, healthcare workers, or other high-risk populations (Halatoko *et al.*, 2020; Mukwege *et al.*, 2021; Uyoga *et al.*, 2021). Apart from epidemiological factors, the difference in reported seroprevalence might be explained by the different types of serological assays used, especially those that are designed to detect a single immunoglobulin G (IgG) antibody, and to their target populations. We recently reported on the challenges of SARS-CoV-2 seroprevalence studies conducted in African countries with commercial tests validated in Europe, the United States, or Asia (Nkuba Ndaye *et al.*, 2021).

We aimed to describe the prevalence of IgG antibodies to SARS-CoV-2 in the general population of Kinshasa in order to understand to what extent the virus has spread after the first epidemic wave.

2.2.2. Methods

2.2.2.1. Study Design and Participants

The 2020 Appui à la Riposte Africaine à l'Epidémie COVID-19 (Support for the African Response to COVID-19 [ARIACOV]) survey was a household-based seroprevalence survey conducted between 22 October 2020 and 8 November 2020 in Kinshasa. The sampling frame used the health divisions of the city. Kinshasa is divided into 35 health zones, which are divided, in turn, into 380 health areas, with an estimated total population of 12 117 417 inhabitants (Système National d'Information Sanitaire [National Health Information System]). A health zone is defined as an operational unit, which supports 100 000 to 150

000 inhabitants and is delimited taking into account the geographical, cultural, and economic accessibility of the population. It could be a geographical space contained within the limits of a territory or an administrative commune comprising a population of approximately 100 000 to 250 000 people in urban areas. Kinshasa is divided in 35 health zones for 26 administrative communes based on the number of population within each administrative commune (unit). A 3-stage design was used to randomly select 292 households (Figure 35). First, 14 of 35 health zones (divided into 2 strata corresponding to the eastern and western regions of the city) were selected with a probability to be selected proportional to the number of households in each zone. Then, within each zone, 3 health areas were randomly selected and finally 8 households were selected within each health zone. To balance the groups, all the residents were invited to participate in the study in 50% of households and, among the remaining 50%, only the residents aged 18 years and older were invited to participate.

During the study, all individuals with a suspicion of COVID-19 infection were referred for polymerase chain reaction (PCR) testing and patient care to the COVID-19 reference center. All staff involved in the study were tested by PCR prior to the survey and followed infection, prevention, and control recommendations. Community-based mobilization for the survey was performed in a 2-step process. The study team met with local leaders and key stakeholders a few weeks prior to the start of the survey and also visited each selected cluster to directly mobilize the community about the survey. Participants were told that the survey was about COVID-19/SARS-CoV-2 and that they would be tested for antibodies if they agreed to participate.

A smartphone application (Epicollect 5; Imperial College, London, UK) was used for listing household members and recording answers from the questionnaires. The individual questionnaires collected socioeconomic (eg, common yard vs single-family home, presence of hand-washing device) and behavioral information (eg, absence from Kinshasa), as well as a history of symptoms associated with COVID-19, history of hospitalization, previous history of SARS-CoV-2 tests (recall period starting March 2020), and contact with patients with COVID-19. Interviews were done in French (official language of the DRC) or in any of the 4 national languages (Kikongo, Lingala, Swahili, or Tshiluba).

Ethics approval was obtained from the Comité d’Ethique de l’Ecole de Santé Publique de Kinshasa (protocol no. ESP/ CE/156/2020). All adults and children (≥ 10 years) were informed about the study objectives and procedures. Adults provided written consent to participate in the study and to be tested for SARS-CoV-2 serology prior to starting the interview. Written parental consent and children assent when aged 10 years or older were obtained prior to enrollment of participants younger than 18 years.

2.2.2.2. Detection of Antibodies to SARS-CoV-2/COVID-19

Venous blood samples (3–5 mL) were collected from eligible participants in a “red-top tube,” which did not contain any additives and transported to the National Institute of Biomedical Research. After centrifugation, serum samples were aliquoted and stored at -20°C until laboratory analysis. Presence of antibodies to SARS-CoV-2 was determined with a previously developed, highly sensitive and specific Luminex-based assay (Luminex Corp, Austin, TX, USA) to simultaneously detect IgG antibodies to 2 viral antigens—that is, recombinant nucleocapsid (NC) and spike (SP) proteins derived from SARS-CoV-2, as previously described ([Ahidjo Ayoubu *et al.*, 2020](#)). Results were expressed as median fluorescence intensity for 100 beads. Cutoff values were determined with receiver operating characteristic curve analysis from a panel of SARS-CoV-2–negative and positive plasma samples consisting of European donors before the COVID-19 pandemic and hospitalized PCR-confirmed patients, respectively ([Ahidjo Ayoubu *et al.*, 2020](#)). Specificity was validated on a panel of 1197 samples from Africa before COVID-19 (99.7% specificity) (Table 31). A sample was considered positive for IgG against SARS-CoV-2 if it reacted simultaneously with NC and SP proteins. As several studies have reported a decrease in antibody levels over time, we considered samples with only 1 of 2 antigens above the threshold as “indeterminate” due to the difficulty to discriminate between antibody decline or the lower specificity of single-antigen reaction, as often reported in samples from Africa ([Sheila F. Lumley *et al.*, 2021](#); [Nkuba Ndaye *et al.*, 2021](#)). Samples with a median immunofluorescence intensity below the cutoff for both antigens were considered negative.

2.2.2.3. Statistical Analysis

Statistical analysis was performed using Stata 16 (StataCorp, College Station, TX, USA). Data were checked and analyzed using the `svyset` commands to take into account the survey

de sign. Descriptive statistics were weighted to take into account the selection probability of the cluster sampling procedure and are presented as proportions with their 95% confidence intervals (CIs) or means with standard deviation. The Pearson’s chi-square test was used to compare categorical descriptive outcomes. The overall prevalence estimate was weighted and age-standardized based on available demographic data (The World, 2019). Multivariate logistic models were used to assess the association between positive serology and key risk factors. Likelihood ratio tests were performed to determine the significance of each factor in the model. To estimate the total number of SARS- CoV-2 infections in the population, we multiplied the weighted, age-standardized seroprevalence by the population of Kinshasa at the time of the survey and divided this number by the number of reported COVID-19 cases detected by reverse transcription– PCR on 19 October 2020 to estimate the infection-to-case ratio.

2.2.3. Results

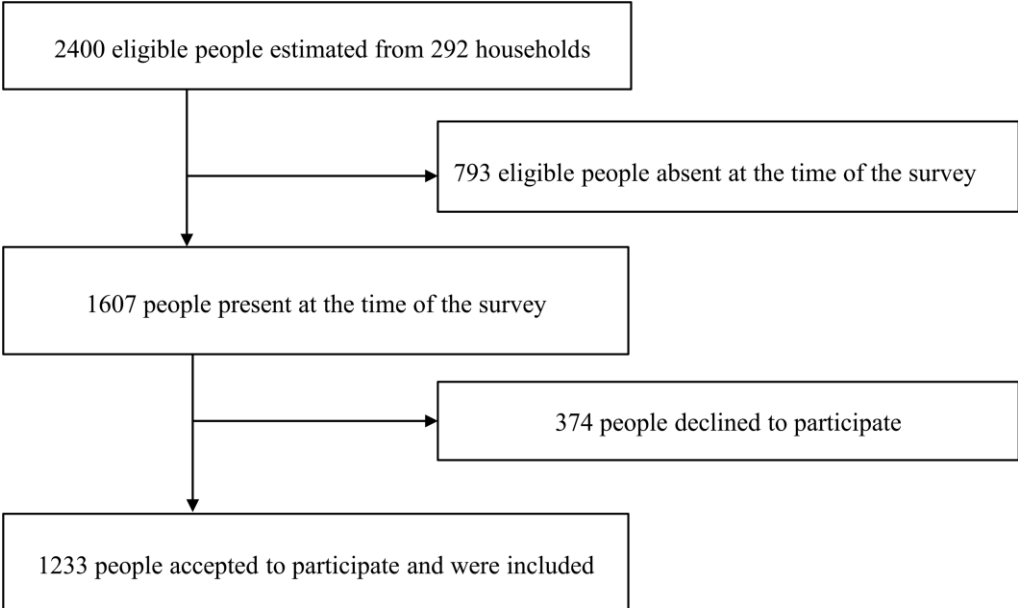


Figure 34. Flowchart of participant inclusion during the SARS-CoV-2 household-based serosurvey.

Abbreviation : SARS-CoV-2, severe acute respiratory syndrome corona-virus 2.

Among 292 randomly selected households from 42 clusters, 2400 individuals were eligible; 1607 were present at the time of the survey and 1233 (76.7%) were included in the final analysis (Figure 34, Table 27). Of these, 1080 provided sufficient and compliant samples that were tested for anti-SARS-CoV-2 IgG antibodies against SP and NC proteins. The mean age of participants was 32.4 ± 19.5 years: 461 (37.4%) were in the 18–39- year age group, 420 (34.1%) in the 40 and older age group, and 352 (28.6%) in the 0–17-year age group; 764 (61.2%) were women. Most participants (72.2%) resided in a common yard and 668 (54.2%) did not have access to handwashing devices at home. In total, 750 (60.8%) declared having completed secondary studies and 349 (28.3%) were pupils or students. Overall, 659 (53.5%) participants were from the eastern part of the city of Kinshasa.

The overall weighted, age-standardized SARS-CoV-2 seroprevalence was 16.6% (95% CI: 14.0–19.5%) with both anti- IgG against SP and NC proteins. In addition, 17.1% (Table 28) of participants were considered as “indeterminate,” as they were positive for SP ($n = 43$; 23.2%) or NC ($n = 142$; 76.8%) antibodies only. Based on the observed prevalence, we estimated that a total of 2 426 406 (Table 32, Figure 35) infections most likely occurred by 19 October 2020 in the general population of Kinshasa for 8290 official reported cases. The ratio of reported cases to estimated infections was 1 :292. Seroprevalence was highest among participants 40 years and older (21.2%; 95% CI: 16.6–26.7%), and lowest among children aged between 0 and 17 years (14.9%; 95% CI: 10.4–20.8%). The observed difference was statistically significant ($P < .05$) between age categories, but was not significant between female and male participants (17.7% [95% CI : 13.9–20.9%] vs 15.7% [95% CI : 11.8–20.7%], respectively) (Table 28).

Table 27. Sociodemographic Characteristics of Participants

	Females	Males	Total
Age, n (%)			
0–17 years	188 (24.9)	164 (34.2)	352 (28.6)
18–39 years	301 (39.9)	160 (33.4)	461 (37.4)
≥40 years	265 (35.2)	155 (33.4)	420 (34.1)
Age, mean ± standard deviation, years	33.4 ± 18.9	30.8 ± 20.2	32.4 ± 19.5
Handwashing device, n (%)			
Present	231 (48.2)	334 (44.3)	565 (45.8)
Absent	248 (51.8)	420 (55.7)	668 (54.2)
Type of residence, n (%)			
Common courtyard	327 (68.3)	563 (74.7)	890 (72.2)
Building	2 (0.4)	1 (0.1)	3 (0.2)
Single-family home	145 (30.3)	184 (24.4)	329 (26.7)
Other	5 (1.0)	6 (0.8)	110 (9.0)
Geographical area, n (%)			
East	410 (54.4)	249 (52.0)	659 (53.5)
West	344 (45.6)	230 (48.0)	574 (46.7)
Number of years residing in Kinshasa, n (%)			
0–4	11 (2.3)	18 (2.4)	29 (2.4)
5–9	6 (1.3)	11 (1.5)	17 (1.4)
10–29	21 (4.4)	22 (2.9)	43 (3.5)
30–69	19 (4.0)	30 (4.0)	49 (4.0)
Always	417 (87.1)	665 (88.2)	1082 (87.8)
Never	5 (1.0)	8 (1.1)	13 (1.1)
Absence from home for >1 month since March 2020, n (%)			
Yes	51 (10.7)	53 (7.0)	104 (8.4)
No	428 (89.4)	701 (93.0)	1129 (91.6)
Number of times absent at night since March 2020, n (%)			
0	397 (82.9)	638 (84.6)	1035 (83.9)
1–4	49 (10.2)	80 (10.6)	129 (10.4)
5–9	10 (2.1)	14 (1.9)	24 (2.0)
≥10	23 (4.8)	22 (2.9)	45 (3.7)
Marital status (participants >15 years), n (%)			
Single	184 (53.6)	273 (44.0)	457(47.4)
Married/living as a couple	139 (40.5)	239 (38.5)	378 (39.2)
Divorced/separated	11 (3.6)	30 (13.6)	41 (4.2)
Widower/widow	9 (2.6)	79 (12.7)	88 (9.1)
Education, n (%)			
None	42 (5.6)	19 (4.0)	61 (4.95)
Primary school	127 (16.8)	98 (20.5)	22 (18.3)
Secondary school	496 (65.8)	254 (53.0)	750 (60.8)
University	89 (11.8)	208 (22.6)	197 (16.0)
Profession, n (%)			
Sales/service	193 (25.6)	52 (10.8)	245 (19.9)
Professional/manager	79 (10.5)	41 (8.6)	120 (9.7)
Pupil/student	189 (25.1)	160 (33.4)	349 (28.3)
Woman/man at home	86 (11.4)	1 (0.2)	87 (7.1)
Construction	1 (0.1)	18 (3.8)	19 (1.5)
Unemployed	126 (16.7)	73 (15.2)	199 (16.1)
Other	80 (10.6)	80 (10.6)	214 (17.4)
Overall, n (%)	754 (61.2)	479 (38.9)	1233

Table 28. Prevalence of SARS-CoV-2 by Sociodemographic Characteristics: Kinshasa, Democratic Republic of the Congo, 2020

	Participants Tested, n	Seropositive ^a		Indeterminate ^b		Seronegative	
		n (%)	95% CI (%)	n (%)	95% CI (%)	n (%)	95% CI (%)
Age							
0–17 years	281	39 (14.9) ^c	10.4–20.8	31 (10.8) ^c	7.2–15.9	211 (75.1) ^c	67.7–80.1
18–39 years	428	53 (13.7) ^c	10.1–18.3	82 (18.5) ^c	15.5–23.2	293 (68.5) ^c	62.3–73.0
≥40 years	371	75 (21.2) ^c	16.6–26.7	72 (19.9) ^c	15.4–25.4	224 (60.4) ^c	52.7–64.8
Geographic area							
East	617	79 (14.9)	11.8–18.8	116 (19.5)	16.0–23.6	422 (68.4)	61.0–70.0
West	463	88 (18.5)	14.6–23.2	69 (14.4)	11.0–18.8	306 (66.1)	61.7–72.1
Gender							
Male	417	59 (15.7)	11.8–20.7	72 (17.0)	13.1–21.9	286 (68.6)	61.4–72.6
Female	663	108 (17.7)	13.9–20.9	113 (17.2)	14.0–20.9	442 (66.7)	61.2–69.9
Handwashing device							
Yes	486	86 (18.2)	14.4–22.7	94 (19.1)	15.2–23.6	306 (63.0)	57.4–67.8
No	594	81 (15.3)	12.0–19.3	91 (15.4)	12.2–19.3	422 (71.0)	64.6–73.6
Type of residence							
Common yard	777	131 (18.0)	14.9–21.6	136 (17.3)	14.3–20.7	510 (65.6)	60.6–68.7
Single-family home	300	36 (13.1)	9.1–18.5	49 (16.9)	12.4–22.6	215 (71.7)	63.5–75.9
Number of years residing in Kinshasa							
0–4	24	3 (18.6)	5.2–48.8	6 (23.0)	8.6–48.9	15 (62.5)	33.0–79.9
5–9	16	0 (0.0)	...	3 (12.5)	3.0–39.5	13 (81.3)	60.5–97.0
10–29	39	7 (18.2)	7.5–37.9	5 (16.7)	5.6–40.4	27 (69.2)	44.0–81.6
30–69	43	12 (30.7)	16.9–49.0	7 (16.4)	6.9–34.5	24 (55.8)	35.4–69.7
Always	945	144 (16.2)	13.5–19.3	160 (16.9)	14.2–19.9	641 (67.8)	35.4–69.7
Never	13	1 (6.2)	0.7–39.3	4 (38.8)	12.4–72.3	8 (61.5)	23.9–82.5
Absence from home for >1 month since March 2020							
Yes	89	10 (13.3)	6.8–24.6	26 (30.4) ^d	20.2–42.9	53 (59.6)	43.8–68.1
No	991	157 (16.9)	14.2–20.0	159 (15.9) ^d	13.4–18.9	675 (68.1)	63.6–70.6
Number of times absent at night since March 2020							
0	917	133 (15.8)	13.1–19.0	148 (16.0)	13.3–19.1	636 (69.4)	64.5–71.8
1–4	109	22 (20.2)	12.8–30.3	27 (24.2)	16.1–34.7	60 (55.0)	44.6–66.1
5–9	21	4 (20.5)	6.6–48.6	5 (25.6)	9.3–53.7	12 (57.1)	29.3–76.8
≥10	33	8 (23.3)	10.6–43.9	5 (17.3)	6.6–38.2	20 (60.6)	45.3–65.6
Marital status (age ≥15 years)							
Single	448	59 (14.6)	11.5–18.4	76 (16.2)	12.5–20.7	313 (69.9)	63.9–74.2
Married/living as a couple	341	61 (19.5)	14.8–25.3	66 (20.2)	15.3–25.7	214 (62.8)	53.9–66.6
Divorced/separated	37	5 (11.5)	3.9–29.6	11 (31.0)	6.7–26.2	19 (51.4)	35.4–76.5
Widower/widow	75	17 (21.8)	12.9–34.5	10 (13.8)	14.8–53.8	48 (64.0)	50.8–76.0
Education (n)							
None	37	8 (25.3)	12.2–45.2	3 (5.9)	1.1–26.6	26 (68.7)	48.8–83.5
Primary school	184	29 (17.4)	11.6–25.3	25 (14.2)	9.1–21.4	130 (68.4)	59.7–76.0
Secondary school	683	103 (16.1)	13.0–19.8	122 (17.9)	14.6–21.6	458 (66.0)	61.6–70.2
University	176	27 (15.8)	10.3–23.5	35 (19.4)	13.3–27.4	114 (64.8)	55.9–72.8
Profession (n)							
Sales/service	220	46 (20.9)	15.2–28.0	58 (26.4)	21.1–35.3	116 (52.7)	43.6–59.2
Professional/manager	111	16 (14.4)	9.2–27.0	19 (17.1)	9.2–27.2	68.5 (68.5)	55.4–77.6
Pupil/student	296	40 (13.5)	10.2–20.3	35 (11.8)	8.0–16.6	221 (74.7)	67.4–79.5
Woman/man at home	81	11 (13.6)	7.7–28.8	11 (13.6)	5.2–20.6	59 (72.8)	60.4–83.9
Construction	19	2 (10.5)	1.7–45.1	3 (15.8)	2.4–33.0	59 (72.8)	50.2–93.7
Unemployed	171	24 (14.0)	10.2–23.9	24 (14.0)	7.7–19.1	123 (71.9)	63.0–79.1
Other	182	28 (15.4)	10.6–23.8	35 (19.2)	14.4–29.1	119 (65.4)	54.1–71.2
Overall	1080	167 (16.6)	14.0–19.5	185 (17.13)	...	728 (67.41)	...

Abbreviations: CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aPresence of antibodies to nucleocapsid and spike proteins.

^bPresence of antibodies to only nucleocapsid or spike protein.

^cSignificant difference at 5%.

^dSignificant difference at 1%.

Table 29. Weighted Proportion of Prevalence of SARS-CoV-2 Stratified by Medical History: Kinshasa, Democratic Republic of the Congo, 2020

	Participants Tested, n	Participants					
		Seropositive ^a		Indeterminate ^b		Seronegative	
		n (%)	95% CI (%)	n (%)	95% CI (%)	n (%)	95% CI (%)
Number of symptoms							
None	324	54 (18.0)	13.3–23.8	48 (15.5)	11.2–21.1	222 (66.5)	59.8–72.6
1 to 2 symptoms	266	42 (16.7)	11.9–22.8	57 (21.2)	15.9–27.7	167 (62.1)	54.9–68.8
3 to 5 symptoms	236	38 (17.5)	12.3–24.2	43 (17.7)	12.6–24.2	155 (64.8)	57.2–71.7
≥5 symptoms	239	30 (13.8)	9.2–20.2	35 (14.1)	9.6–20.2	174 (72.1)	64.7–78.4
Symptoms							
Fever							
No	692	113 (17.6)	14.3–21.3	123 (18.1)	14.9–21.9	456 (64.3)	59.9–68.5
Yes	388	54 (14.8)	11.0–19.8	62 (15.3)	11.5–20.1	272 (69.9)	64.1–75.1
Chills							
No	829	142 (18.3)	15.3–21.8	147 (17.7)	14.7–21.0	540 (64.0)	60.0–67.9
Yes	244	24 (11.2)	7.1–17.1	37 (15.3)	10.6–21.7	183 (73.5)	66.2–79.7
Fatigue/asthenia							
No	844	132 (16.6)	13.8–20.0	152 (17.7)	14.9–21.0	560 (65.6)	61.7–69.4
Yes	232	34 (16.4)	11.3–23.3	32 (14.7)	9.9–21.3	166 (69.9)	61.1–75.7
Muscle pain (myalgia)							
No	832	118 (14.6)	11.9–17.8	148 (17.9)	15.0–21.3	566 (67.5)	63.6–71.2
Yes	240	48 (23.7)	17.6–31.1	36 (14.7)	10.1–20.9	156 (61.7)	53.9–68.9
Sore throat							
No	941	148 (17.0)	14.2–20.2	158 (16.7)	14.0–19.8	635 (66.3)	62.5–69.8
Yes	139	19 (14.0)	8.4–22.4	27 (19.7)	13.0–28.8	93 (66.3)	56.3–75.0
Cough							
No	791	128 (17.2)	14.2–20.7	133 (17.0)	14.1–20.5	530 (65.7)	61.6–69.6
Yes	289	39 (14.8)	10.4–20.7	52 (17.7)	12.7–23.2	198 (67.8)	61.0–74.0
Runny nose (rhinorrhea)							
No	770	122 (16.7)	13.7–20.2	138 (18.2)	15.2–21.8	510 (65.1)	60.9–69.0
Yes	310	45 (16.3)	11.8–22.1	47 (14.3)	10.3–19.5	218 (69.4)	62.9–75.3
Breathing difficulties							
No	1037	163 (16.9)	14.3–19.9	176 (16.9)	14.3–19.9	698 (66.2)	62.6–69.5
Yes	43	4 (8.2)	2.6–23.4	9 (22.5)	10.8–40.9	30 (69.3)	51.0–83.0
Loss of taste and smell (ageusia/anosmia)							
No	929	144 (16.7)	13.9–19.9	162 (14.5)	14.7–20.6	623 (65.9)	62.1–69.5
Yes	141	23 (16.1)	10.2–24.5	23 (15.3)	9.7–23.3	105 (68.6)	59.2–76.7
Chest pain							
No	849	160 (16.8)	14.2–19.9	171 (16.8)	14.2–19.8	678 (66.4)	62.7–69.8
Yes	160	7 (13.3)	6.0–27.2	14 (21.4)	12.1–25.0	50 (65.3)	50.9–77.3
Other respiratory symptoms							
No	1066	12 (16.6)	14.0–19.5	182 (17.1)	14.5–20.0	719 (66.3)	62.8–69.6
Yes	14	2 (15.4)	3.3–49.0	3 (21.4)	5.9–54.3	9 (63.2)	32.5–86.0
Anorexia							
No	917	146 (16.8)	14.1–20.0	162 (17.8)	15.0–21.0	609 (65.4)	61.6–69.0
Yes	163	21 (15.4)	9.6–23.7	23 (13.3)	8.3–20.7	119 (71.3)	62.2–79.0
Headache							
No	690	108 (17.2)	13.9–21.0	121 (17.5)	14.3–21.2	461 (65.3)	60.9–69.5
Yes	390	59 (15.6)	11.7–20.4	64 (16.4)	12.5–21.3	267 (68.0)	62.2–73.3
Nausea/vomiting							
No	959	156 (17.4)	14.7–20.6	159 (16.6)	14.0–19.7	644 (66.0)	62.3–69.5
Yes	121	11 (9.7)	4.9–18.3	26 (21.5)	14.0–31.6	84 (68.8)	58.1–77.9
Abdominal pain							
No	862	193 (17.6)	14.7–21.0	146 (17.1)	14.2–20.3	574 (65.3)	61.4–69.1
Yes	218	25 (12.3)	7.8–18.8	39 (17.5)	12.2–24.3	154 (70.3)	62.4–77.0
Diarrhea							
No	956	103 (16.5)	13.8–19.6	161 (16.9)	14.2–19.9	649 (66.7)	63.0–70.2
Yes	124	21 (17.6)	10.9–27.1	24 (19.1)	12.2–28.7	79 (63.3)	52.8–72.7
Hospitalization							
No	1024	153 (16.0) [§]	13.4–19.0	177 (17.3)	14.7–20.3	694 (66.7)	38.6–70.0
Yes	52	14 (29.8) [§]	17.0–46.8	8 (15.5)	7.0–30.7	30 (54.8)	63.1–70.1

Abbreviations : CI, confidence interval ; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aPresence of antibodies to nucleocapsid and spike proteins.

^bPresence of antibodies to only nucleocapsid or spike protein.

[§]Significant difference at 5%.

Seroprevalence was higher among participants from the western region of Kinshasa (18.5%; 95% CI: 14.6–23.2) than among those from the eastern area (14.9%; 95% CI: 11.8–18.8%), but the difference was not significant. Among participants who reported the type of residence, there was no significant difference between residents with a common yard (18.0%; 95% CI: 14.9–21.6%) and those who resided in a single-family home (13.1%; 95% CI: 9.1–18.5%). We investigated the influence of being absent at home on SARS-CoV-2 seroprevalence and observed no significant difference with those still present since March 2020 (13.3% [95% CI: 6.8–4.6%] vs 16.9% [95% CI: 14.2–20.0%], respectively).

Of the 1080 participants with blood samples, 741 (68.6%) reported having at least 1 of the 16 symptoms reported to be associated with COVID-19 infection. Among participants who reported no symptoms in the past 8 months, 18.8% (95% CI: 13.3–23.8%) were positive for SARS-CoV-2 IgG SP and NC proteins, but no significant differences were observed among those who reported symptoms. We did not observe associations with a single or a combination of clinical symptoms and seropositivity. We also assessed whether hospitalization could be associated with seroprevalence and observed that the proportion of people who admitted to have been hospitalized before the survey and positive for anti-SARS-CoV-2 antibodies (29.8%; 95% CI: 17.0–46.8%) was significantly higher compared with those who were not hospitalized (16.0%; 95% CI: 13.4–19.0%)(Table 29). None of the households reported deaths with symptoms related to COVID-19.

We then performed multivariate analysis for all parameters with a significant difference of seroprevalence between groups, but no significant association between age, gender, and hospitalization with seroprevalence was found (Table 30).

Table 30. Association Between Antibodies to SARS-CoV-2 and Risk Factors: Multivariate Logistic Model, Kinshasa, Democratic Republic of the Congo, 2020

Variable	Seropositive ^a -Participants, n (%)	Univariate Odds Ratio (95% CI)	Multivariate Odds Ratio (95% CI)
Gender			
Male	59 (14.1)	1	1
Female	108 (16.2)	1.11 (.73–1.68)	1.12 (.73–1.7)
Age			
0–17 years	39(13.9)	1	1
18–39 years	53 (12.4)	.91 (.53–1.55)	.89 (.52–2.52)
≥40 years	75 (20.2)	1.54 (.92–2.57)	1.51 (.91–2.52)
Hospitalization			
Yes	14 (26.9)	1	1
No	153 (14.9)	0.59 (0.30–1.16)	0.60 (0.31–1.17)

2.2.4. Discussion

To our knowledge, this is the first serological survey conducted in the general population of Kinshasa after the first wave of the COVID-19 pandemic (March to September 2020). The overall prevalence of anti-SARS-CoV-2 antibodies was 16.6%. Extrapolation to the entire population showed that approximately 2.4 million infections occurred between March and October 2020 in contrast to the 8290 PCR-confirmed cases reported during the same period. We estimated that most cases went unnoticed, with only 1 case detected for every 292 infections.

Seroprevalence in the DRC was higher than that reported in India, Brazil, Switzerland, and Zambia (Hallal *et al.*, 2020; Mulenga *et al.*, 2021; Murhekar *et al.*, 2021; Stringhini *et al.*, 2020), but was probably linked to the different age structure or to the early stage of the COVID-19 pandemic, as was the case in Zambia (Mulenga *et al.*, 2021). Several other countries in Africa have reported varying seroprevalences (eg, the prevalence was lower in Kenya, Togo, and Malawi (Chibwana *et al.*, 2020; Halatoko *et al.*, 2020; Uyoga *et al.*, 2021), but higher in Niger, the Ivory Coast, and South Sudan (Majiya *et al.*, 2020; Milleliri *et al.*, 2021; Wiens *et al.*, 2021). This variability could be explained, first, by the fact that most of these studies were carried out in specific populations and, second, apart from the Malawi study, all the other studies reported a seroprevalence using positivity against a single antigen (SP or NC).

Although we observed a trend among age groups, seroprevalence was not significantly associated with age groups in our survey. Indeed, several studies have reported a different distribution of seroprevalence according to age, whereas others did not observe age-related differences—for example, in India, Brazil, and Zambia, seroprevalence was similar between age groups (Hallal *et al.*, 2020; Mulenga *et al.*, 2021; Murhekar *et al.*, 2021). However, a study conducted in Iran reported that the prevalence of COVID-19/SARS-CoV-2 varied by age group (Salehi *et al.*, 2017). In Switzerland, seroprevalence was significantly lower among young children (5–9 years) and older people (≥ 65 years) than for other age groups (Stringhini *et al.*, 2020). Only limited data on the kinetics of antibodies in children are available and most tests were validated on samples of adults with symptoms. However,

antibody titers and kinetics in children exposed to SARS-CoV-2 are most likely similar to those in adults (Roarty *et al.*, 2020).

On the basis of our pilot study that showed a low agreement between the results of commercially available antibody-detection assays validated in Europe, the United States, or Asia on African samples, we recommend the use of a combination of serological tests, targeting 2 or more independent antigens in this context (Nkuba Ndaye *et al.*, 2021). This is even more important with the advent of mass vaccination. Therefore, assuming that seropositivity against SP and NC proteins is evidence of true seroconversion, we estimated the seroprevalence of antibodies against SARS-CoV-2 at 16.6%. During the first wave (March to September), the majority of notified PCR-confirmed cases came from the western health zone of Kinshasa, but our study showed that seroprevalence was almost similar between the 2 geographic regions studied, thus suggesting that COVID-19 had spread throughout the entire city despite early government actions. Nevertheless, this high seroprevalence was not accompanied by higher mortality rates or saturation of hospital services. The SARS-CoV-2 virus is known for its ability to be transmitted to all ages, but the risk of developing a severe form increases with age and other risk factors, including obesity among younger individuals (Brodin, 2021; Dennis *et al.*, 2020). Indeed, the age pyramid in the DRC is made up of a large base of young people, with a small number of the elderly, and people aged 65 years and older represent only 3% of the total population (The World, 2019).

In many studies, seroprevalence is determined using only 1 of the 2 major SARS-CoV-2 antigens (Halatoko *et al.*, 2020; Mukwege *et al.*, 2021; Murhekar *et al.*, 2021; Selvaraju *et al.*, 2021; Uyoga *et al.*, 2021). This may lead to an overestimation due to cross-reactivity of the SP or NC antibodies against other viral antigens (Yaniv Lustig *et al.*, 2020) or underestimation by antibody waning (Sheila F. Lumley *et al.*, 2021). Cross-reactivity can occur with common circulating coronaviruses, as well as other viruses such as dengue (Yaniv Lustig *et al.*, 2020). In contrast to anti-SP IgG antibodies, which are sustained over time, the half-life of the SARS-CoV-2 anti-NC IgG response seems to be shorter (Sheila F. Lumley *et al.*, 2021). Seroprevalence of antibodies against the SP or NC proteins only was 17.1% in our study. The overall seroprevalence in Kinshasa combining all positive and indeterminate groups could thus be higher and reach 32.6%. Seroprevalence based on positivity to 2 different SARS-CoV-2 antigens thus provides most likely minimal estimates,

and it is probable that a proportion of the participants with antibodies against a single antigen also represented individuals who had a previous SARS-CoV-2 infection. Nevertheless, most of the population from Kinshasa remains not infected as yet and it is hoped that the spread of SARS-CoV-2 can be maintained until the herd immunity threshold is achieved. This estimated threshold is approximately 50% to 67%, but it could be reached faster by vaccination rather than natural immunization (Fontanet *et al.*, 2020; Randolph *et al.*, 2020). Evidence of antibodies was found among participants who did not report having symptoms in the past 8 months. Similar findings have been reported in many countries (Mulenga *et al.*, 2021; Oran *et al.*, 2021). These data underline the importance of testing asymptomatic individuals before traveling by air or other means of transport connecting different regions, even if they did not report any notion of exposure to SARS-CoV-2.

Our study has several limitations. Based on the assumption that 50% of the population are aged 18 years and younger, we invited all residents from 50% of the households to participate in the study, while only people aged 18 and older were invited to participate in the remaining 50% of households. Participation was only 50% and more women were included, which may limit the generalizability of our findings. Seroprevalence in age groups should be interpreted with caution as the age adjustment was done based on the 2019 population estimation (The World, 2019). Additionally, COVID-19–related symptoms that participants had developed in the previous 8 months were reported retrospectively, resulting in a probable recall bias. We also probably missed some recent infections because we only tested the presence of IgG antibodies, as illustrated by the lower sensitivity of our assay on a panel of samples collected between 1 and 30 days after symptom onset, suggesting a possible underestimation of recent infections.

2.2.5. Conclusion

The results of the first household SARS-CoV-2 serosurvey in Kinshasa show a high seroprevalence and spread in both the eastern and western regions of the city, illustrating that most cases were undiagnosed. These results provide an excellent picture of the extent of the COVID-19 pandemic in Kinshasa after the peak of the first wave, as well as lessons for adjusting the countermeasures. The country is now facing the second wave, which is apparently more contagious than the first one. Our findings therefore support strengthening

of the testing capacity for both symptomatic and asymptomatic individuals, strict application of nonpharmaceutical measures, and improvement in the management of severe cases. Finally, we provide evidence of the value of conducting serological surveys at regular intervals in both extended areas of Kinshasa and in other regions of the DRC to better understand the trend of the pandemic, identify the population categories at highest risk for clinical complications, and estimate the herd immunity threshold in order to use vaccines in a cost-effective manner.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The contents of this document are the authors' opinions and do not necessarily reflect those of the Ministry of Public Health of the DRC government.

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2.2.6. Supplementary materials

Supplementary material

Table 31. Number and percentage of samples collected before the COVID-19 epidemic to evaluate the specificity of the assay on African samples

Country	Year	Tested (n)	NC (n+)	SP (n+)	NC+SP (n+)
DRC	2018	92	2	2	0
	1997	92	2	4	0
Cameroon	2012	184	4	13	
Guinea	2018	829	35	20	2
Total	1197		43	39	3
Specificity			96.5%	96.8%	99.7%

Table 32. Estimated number of SARS-CoV-2 infections in Kinshasa, DRC (March-October, 2020)

	Kinshasa
2020 population projection ^a , n	12 117 417
Estimated number of SARS-CoV-2 infection ^b , n	2 426 407
Official reported cases, n	8290
Infections/case ratio	292:1

^a; population projection, DRC National Health Information System (DHIS2) <https://snisrdc.com/>.

^b; Calculated considering the prevalence of individuals seropositive with both anti-IgG against spike and nucleocapsid SARS-CoV-2 antigens.

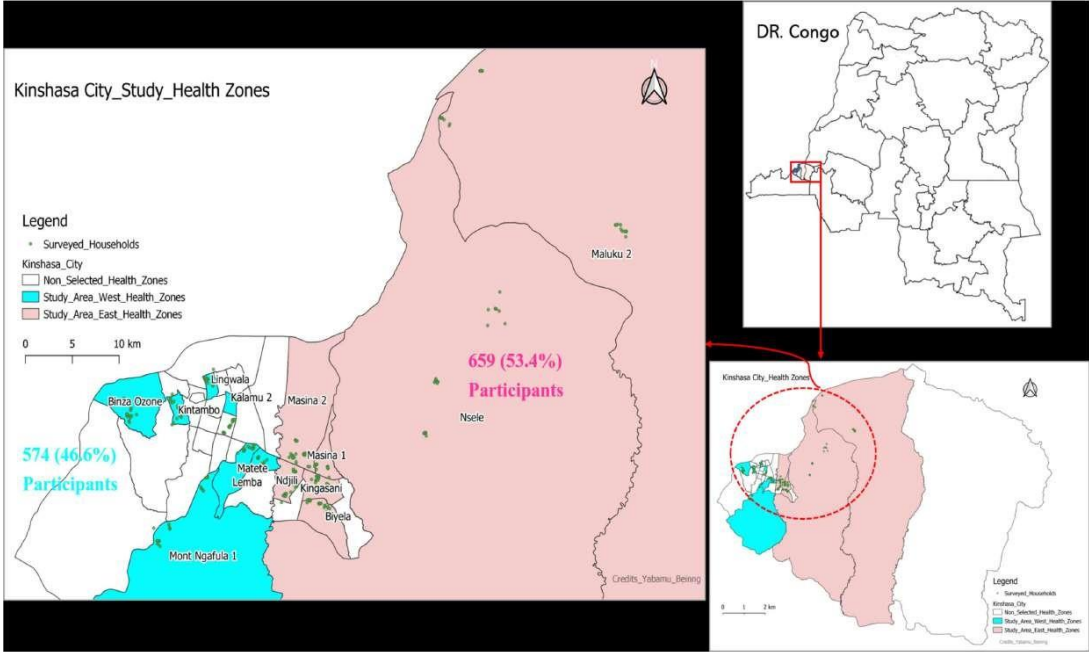


Figure 35. Study area and participants proportions.

General discussion and conclusion

Discussion

At the beginning of our Ph.D. project, the initial aim was to work mainly on questions related to EVD, in particular the impact of the new tools used during the tenth epidemic in the DRC. However, the COVID-19 pandemic declared in March 2020 disrupted the established program and allowed us to work on issues related to the newly identified virus SARS-CoV-2 (Cucinotta *et al.*, 2020; Del Rio *et al.*, 2020). Therefore, our thesis aimed to characterize the human humoral immune response to Ebola virus in order to understand the place occupied by pauci-symptomatic EVD infection during an epidemic and to evaluate the impact of new treatments used during the 10th outbreak of EVD in the DRC. We also aimed to understand the true extent of SARS-CoV-2 in Kinshasa, the capital of the country, and provide these findings and information to public health authorities for policy change and adaptation of outbreak response to real needs.

The main results of the thesis are:

- (i). We have found 2.3% of samples positive for anti-IgG antibodies to at least two EBOV antigens using Luminex® method among suspected EVD cases discharged as negative during the 10th EVD outbreak in North Kivu and Ituri province in the DRC suggesting that these individuals are missing EVD cases.
- (ii). Among 358 EVD survivors followed-up for up to 36 months, 23.7% of them were seronegative for at least two antigens at the discharge from the ETC endpoint. Antibody trend showed an up-to-down trend but with a continuous decrease in an overall linear evolution. We have observed that survivors who received specific treatments during the 10th EVD outbreak in the DRC had a faster fall in antibody concentration than those who did not received specific EVD treatments in Guinea during the 2014-2016 EVD outbreak (POSTEBOGUI Study). Finally, antibody titer falls faster in the group of survivors treated with Ansumimab compared to other treatment groups.
- (iii). Age adjusted seroprevalence of SARS-CoV-2 in Kinshasa after the first wave of the pandemic was 16.6% showing a wide spread of the disease compared to the official

number of reported cases; the seroprevalence increased significantly age and the infection appears to have spread evenly between the western and eastern regions of the city of Kinshasa, in stark contrast to the reported data showing a very high prevalence of the disease in the western part of the city.

In this chapter we will outline our discussion in the context of emerging diseases. We will first discuss the new findings made in this thesis on the serological characteristics of individuals infected with the Ebola virus and SARS-CoV-2 and then we will see how this information can contribute to the fight against emerging infectious diseases.

1.1. Understanding pauci-symptomatic Ebola virus infection during Ebola outbreak

1.1.1. Discussion of the results

We have reported 2.3% of missed EVD cases and have estimated that 1,314 undiagnosed EBOV infection may occurred during the 10th EVD outbreak in the DRC among suspected EVD cases discharged as negative after two consecutive RT-PCR tests. In fact, EBOV is known to cause severe disease in humans and other animals such as antelopes, monkeys, and pigs, suggesting that asymptomatic or pauci-symptomatic disease is an anecdotal reality. Because of this, the concept of asymptomatic or pauci-symptomatic infection has long been discussed but poorly understood or demonstrated. However, recently published data have highlighted possible unidentified infection in asymptomatic or pauci-symptomatic contacts of confirmed EVD cases. For example, the studies conducted during the Ebola outbreak in West Africa between 2014 and 2016 reported variable positive serological prevalence. Higher proportions than those observed in our study were recorded among high-risk contacts in Sierra Leone (7.5%) ([Halfmann *et al.*, 2019](#)) and Guinea (4%) ([Diallo *et al.*, 2019](#)). In another study conducted in households members of confirmed EVD cases, the prevalence of asymptomatic infection was close to ours at 2.6% ([Glynn *et al.*, 2017](#)). In a cohort of survivors and their contacts in Liberia, 107(3%) individuals were selected as positive among contacts of EVD survivors based on a serological test that detected antibodies against the Ebola virus glycoprotein ([Kelly *et al.*, 2022](#)).

Our results were obtained using restrictive positivity criteria based in particular on the profile of Guinean survivors, where positivity was determined by a subject's reactivity to at least two of the three Ebola virus antigens included in our analysis panel that contains nucleoprotein, glycoprotein and viral protein 40 antigens (Diallo *et al.*, 2021). These results strengthen our knowledge and understanding of the asymptomatic or pauci-symptomatic clinical of Ebola virus infection and further demonstrate the evolution and adaptation of Ebola virus over time. Indeed, after more than 40 years of involvement in multiple epidemics since its discovery in 1976 in the DRC and Sudan, EBOV appears to be increasingly adapted to humans, causing an admittedly but still low proportion of less severe illness. Therefore, we can hypothesize that the virus may have reached the stage of an ongoing epidemic or endemic host-to-host transmission of the disease in the new host population. This could be due to the multiple cycles that the virus goes through in its natural host, but also to the process of host switching during animal diseases or epidemics. The steps involved in the emergence of host-switching viruses show the host and viral processes that may be involved in the process of transmission and adaptation (Morens *et al.*, 2004; Parrish *et al.*, 2008). This adaptation of Ebola to its new host could be one of the reasons of high frequency of Ebola outbreaks that have been reported in recent years in particular in the DRC. However, one of the ways to test this hypothesis is to conduct serological surveys in remote areas in order to highlight the possible existence of unrecognized epidemics.

It is becoming increasingly clear that an outbreak of EVD can start from a cured person carrying the quiescent virus in privileged immunological sites such as the aqueous humor or the testes. Examples include genomic reports of Ebola viruses responsible for recent outbreaks and known relapses in the DRC and Guinea (Keita *et al.*, 2021; Mbala-Kingebeni *et al.*, 2022; Mbala-Kingebeni *et al.*, 2021). In this context, serological analysis can be used to assess missing EVD cases in clinically suspected EVD cases that came out negative after RT-PCR testing, but also household contacts of confirmed EVD cases who have developed or not symptoms. Therefore, people who test positive for EVD by serologic testing should be followed up, like survivors to prevent recurrence of EBOV and possible transmission of the virus to others. Thus, it is important to carefully select the test to be used and in particular the positivity criteria that can optimize sensitivity and specificity for EBOV.

1.1.2. Perspectives

Although the serological results provided the necessary information that allowed us to better understand the asymptomatic or pauci-symptomatic infection caused by the Ebola virus, they still have some limitations. It cannot be excluded that the antibodies found in the suspected EVD of the 10th outbreak were older cases. Furthermore, it cannot be said that people with antibodies to the Ebola virus are also protected. Therefore, it seems necessary to further our research in this area. First, it will be useful to follow the suspected EVDs declared positive by serological tests, in particular to document the persistence of the virus in the biological fluids of this group. Second, we will study the cellular immunological response of the suspects compared to that of the survivors and negative controls. Finally, we believe it is useful to evaluate the functionality of binding antibodies detected by serological assays in sero-neutralization.

1.2. Understanding longitudinal antibody response in Ebola survivors after specific anti-Ebola treatment

1.2.1. Discussion of the results

We followed up to 36 months for certain participants and reported for the first time the trend of antibody kinetics in EVD survivors treated with anti-Ebola molecules compared to other studies in which survivors did not receive such drugs. (Davis *et al.*, 2019; Diallo *et al.*, 2021; Keita *et al.*, 2019). Participants received three monoclonal antibodies (ZMapp, Ansumab and Inmazeb) and an antiviral molecule called Remdesivir. Among these, two molecules showed an advantage in terms of survival rates and were subsequently approved by the FDA as a treatment for EBOV. Indeed, we observed a high percentage (23.7%) of participants seronegative for at least two antigens at the exit of the ETC endpoint, while several studies that have described the natural history of EVD reported that antibodies with isotype G (IgG) are detected early, generally in the first week after the onset of symptoms (Ploquin *et al.*, 2018; Rimoin *et al.*, 2018; Rowe *et al.*, 1999). In our report, the median from symptom onset to the day of discharge was 20 days, and the interval was ≤ 15 days only for four participants. Therefore, the specific treatments received may be the cause of the impaired antibody production that we observed. It is therefore extremely important to assess the immune status of treated survivors when they leave ETCs and to ensure regular

follow-up to detect possible viral persistence to reduce the risk of a short-term relapse, as occurred in DRC during the 10th EVD outbreak. Additionally, abnormally low antibody levels were observed in two EVD survivors from the 10th outbreak in the DRC who developed viral meningitis after being discharged as cured. Also, they have been treated with monoclonal antibodies at the first episode of the EBOV infection (data in preparation for publication).

Compared to survivors from Guinea, our cohort of survivors showed a rapid decline in antibodies over time with a significantly lower probability of being sero-positive to EBOV antigens at month 36 in all treatment groups and we saw that the group of survivors who received Ansumab lose their antibodies rapidly compare to other treatment groups. In fact, we believe that the treatments received may be the cause of this rapid decline in antibodies in our cohort, although we cannot exclude the influence of other factors such as age, viral load in the acute phase and, in some of them, the vaccine. Animal experiments have shown that antibody production can be disrupted by passive immunization. In mice, it has been suggested that maternal antibodies inhibit antibody production by internalizing maternal antibodies by antigen-presenting cells and suppressing antigenic epitopes of B lymphocytes (Crowe *et al.*, 2001; Siegrist, 2003). In macaques, monoclonal antibody therapies were associated with persistence of Ebola virus in nerve cells (J. Liu *et al.*, 2022). Studies conducted on humans have shown the same trend, for example a retrospective cohort study in the US in patients with COVID-19 treated with monoclonal antibodies showed inhibition of the endogenous humoral response among them (Kim *et al.*, 2022).

In the context of recurrent EVD epidemics in Africa in general and in the DRC in particular, survivors play an important role in these emergencies. Our findings are very important in that they underscore the need to continue to care for EVD survivors, not only to facilitate their reintegration into the community, but also to establish a schedule for clinical, biological, and psychological follow-up. In the DRC, the national program for survivors of the 10th Ebola epidemic was established in collaboration with several partners and stakeholders involved in the response to the epidemic. Coordinated collaboration among partners and the government is needed to converge the efforts and organize activities that will benefit survivors and the community, but also allow scientists to answer the myriad questions about EVD and survivors that remain poorly understood.

1.2.2. Perspectives

As a future perspective, we propose to continue research on the kinetics of antibodies in Ebola survivors, both to better understand the impact of long-term treatments in this patient population and to measure the degree to which survivors are protected from reinfection and/or relapse. As mentioned earlier, monoclonal antibodies seem to have an effect on the humoral immune response but not on the cellular response. It is therefore very important to evaluate this aspect in our cohort to complete our knowledge on this topic and eventually propose appropriate public health measures. Additionally, it's critical to assess EBOV glycoprotein-based vaccines to determine whether they can enhance the endogenous immune response in EVD treated survivors.

1.3. Determine the real extent of SARS-CoV-2 infection in African setting: case of Kinshasa/DRC

1.3.1. Discussion of the results

After the first wave of COVID-19, which was formally recognized as present in the DRC on March 10, 2020 ([Juma *et al.*, 2020](#)), we had done a seroprevalence survey of SARS-CoV-2 in the general population. We estimated that 2,426,406 infections took place during the first six months of the virus's circulation in the city of Kinshasa based on the age-adjusted prevalence, which was 16.6%. These findings were among the first seroprevalence studies to be carried out in the general population in African countries ([Mulenga *et al.*, 2021](#); [Wiens *et al.*, 2021](#)), where the number of confirmed cases reported was very low compared to other continents but also compared to prediction models which announced over 75 million cases during the first year of virus circulation ([Patrick G. T. Walker *et al.*, 2020](#); [Chad R. Wells *et al.*, 2020](#)). However, other investigations from the same time period have found wildly varying frequencies, sometimes with incredibly low prevalence ([Chibwana *et al.*, 2020](#); [Halatoko *et al.*, 2020](#); [Uyoga *et al.*, 2021](#)) and other times with incredibly high prevalence ([Majiya *et al.*, 2020](#); [Milleliri *et al.*, 2021](#); [Wiens *et al.*, 2021](#)). This variation may be caused by the fact that the majority of these investigations were carried out in a particular community, but it may also be because the bulk of the tests utilized in these researches were developed based on a single SARS-CoV-2 antigen, which can affect how well these tests perform. In fact, in many reports, SARS-CoV-2 antibodies have been known

to cross react with antigens from other pathogens such as Dengue virus and *Plasmodium* (Lapidus *et al.*, 2021; Y. Lustig *et al.*, 2021; Steinhardt *et al.*, 2021). In our study, we compared two serological tests: an ELISA test and a rapid test, both based on the spike protein of SARS-CoV-2, and the prevalence determined using the same samples was 36% and 30%, respectively, showing poor agreement between tests and illustrating how challenging it can be to interpret serological analysis in the African context, where several other pathogens are endemic. In order to have a serological test with high precision, we developed a multiplex test during the first national containment in France which took place from March 17 to May 11, 2020 (Wikipedia, 2022). We used plasma samples taken from confirmed COVID-19 patients hospitalized at the Centre Hospitalier Universitaire (CHU) de Montpellier. After evaluating our assay, we found an accuracy of 100% (95% CI: 100-100) for spike protein and 99.9% (95% CI: 99.7-100) for nucleoprotein. After setting the cut-off values for spike and nucleoprotein antigens, we considered positive any plasma that reacted simultaneously to the two antigens tested. Indeed, this criterion made it possible to determine a seroprevalence close to the true magnitude of SARS-CoV-2 in Kinshasa, since this criterion would reduce the overestimation due to cross-reactions. Additionally, studies done after the second, third, and fourth COVID-19 waves also revealed that seroprevalence had increased by a factor of two to three compared to that documented after the first wave, topping 50% in Mali, Cameroon, and Zimbabwe (Fryatt *et al.*, 2021; Ndongo *et al.*, 2022; Sagara *et al.*, 2021).

Our results, together with those of other reports of the seroprevalence of SARS-CoV-2 in African countries, allowed us to draw two important conclusions:

(1) SARS-CoV-2 spread widely in Africa after the first wave of the pandemic and even more after the following waves reached more than 50% in several countries, despite the containment measures taken by the governments of these countries. It is therefore clear that the data communicated by the official bodies, based on molecular confirmation of the infection, are largely underestimated and one study evaluates the average underestimation factor at 8.5 (Thenon *et al.*, 2022). Indeed, this underestimation also reveals the limitations of health systems in several African countries, including the DRC. These limitations refer to the weak diagnostic capacity (Figure 36), the lack of appropriate hospitalization facilities, and the weakness of conducting community-level surveillance for COVID-19 (Sachs *et al.*,

2022; Thenon *et al.*, 2022). In the DRC, the average daily SARS-CoV-2 RT-PCR testing rate is 0.01/1000 inhabitants, ranging from 30 to 5000 tests per day for a population of more than 90 million individuals, with an average positivity of 7.86% taking in account the period from March 11, 2020 to September 23, 2022 (Foundation for Innovative New Diagnostics (FIND), 2021).



Figure 36. SARS-COV-2 tests performed against target values worldwide.

Here is the daily average rate of SARS-CoV-2 RT-PCR tests in six regions of the World. The dashed line shows the target number of tests that must be done. In African countries did not reach their established testing targets (Foundation for Innovative New Diagnostics (FIND), 2021).

(2) Although the reported number of COVID-19 cases and deaths was underestimated, we believe that the number of severe forms of COVID-19 and the number of deaths were lower

than those reported from other regions of the World such as America and Europe. In contrast to other continents, Africa very rarely reported hospital overcrowding scenarios or simultaneous deaths of multiple patients. We think that the age pyramid in Africa, which is distinguished by a relatively high density of young individuals < 40 years old (only 3% to 6% \geq 60 years old), may explain the difference in the clinical presentation of the pandemic between Africa and other continents. Age has been acknowledged as a significant factor that is related to the severity of the COVID-19 condition. Furthermore, the severe form of COVID-19 has a strong correlation with non-transmitted metabolic disorders as diabetes and obesity as well as cardiovascular diseases that are also significantly associated with age (Bepouka *et al.*, 2022; Chidambaram *et al.*, 2020; Williamson *et al.*, 2020). Studies have not been able to show that other factors, such as climatic conditions (S. Chen *et al.*, 2021; Heibati *et al.*, 2021) and potential preexisting partial immunity due to exposure to other coronaviruses (Abbasi, 2022; Wratil *et al.*, 2021), could have a significant influence on the low severity of COVID-19 disease shown in Africa.

Ultimately, the SARS-Cov-2 pandemic not only disrupted the movement of people and their properties, but also reminded us of bad memories, such as arms race that nations faced before and after the first World War. From the production and distribution of face masks to the production and distribution of vaccines, nations have been individualistic, despite the slogans of universal solidarity and globalization. Countries with limited resources, such as African countries, have struggled to obtain equipment and vaccines to fight the pandemic. However, seroprevalence results, such as those we reported in the DRC, could help decision makers to take effective and efficient measures to combat the pandemic, by vaccinating people at risk of the severe form of the disease in a context of shortage of vaccines.

1.3.2. Perspectives

We have seen that the results of SARS-CoV-2 serology also seem to depend on the type of tests used and in particular the viral antigens linked to these tests. The way these antigens are produced and purified, but also the nucleotide sequences used, can influence the final results. Thus, in this context, we have started a meta-analysis of seroprevalence articles published in Africa and will focus our analyzes on the types of antigens and tests used in these different studies. It is also important to understand the spread of the disease following

the introduction of the vaccine and the spread of highly contagious variants such as the Omicron variant. Other general population surveys are therefore very important.

1.4. Conclusion

This work, carried out as part of our thesis project, has shown that patients infected with EBOV can develop a pauci-symptomatic form and sometimes not be diagnosed by RT-PCR, perhaps because they have been tested after the viraemic period or they have low viral load. We also noted that the specific new treatments used during the 10th Ebola outbreak in the DRC appear to be associated with the rapid decline in antibodies over time, although we cannot rule out the influence of other factors. Our work on SARS-CoV-2 has shown that the virus has spread widely in Kinshasa, contrary to officially published data. The very high frequency of Ebola epidemics and the occurrence of the SARS-CoV-2 pandemic therefore demonstrate the need to continue research on the human-animal interface and to deepen the understanding of the human immune response against these pathogens on the means of prevention such as vaccines and the various treatments that have already been developed and are under development.

Article associated with the thesis work

- 1) **Nkuba Ndaye A**, Hoxha A, Madinga J, Mariën J, Peeters M, Leendertz FH, Ahuka Mundeke S, Ariën KK, Muyembe Tanfumu JJ, Mbala Kingebeni P, Vanlerberghe V. *Challenges in interpreting SARS-CoV-2 serological results in African countries*. Lancet Glob Health. 2021 May;9(5):e588-e589. doi: 10.1016/S2214-109X(21)00060-7. Epub 2021 Feb 17. Erratum in: Lancet Glob Health. 2021 Mar 8;: PMID: 33609481; PMCID: PMC7906714.
- 2) **Nkuba AN**, Makiala SM, Guichet E, Tshiminyi PM, Bazitama YM, Yambayamba MK, Kazenza BM, Kabeya TM, Matungulu EB, Baketana LK, Mitongo NM, Thaurignac G, Leendertz FH, Vanlerberghe V, Pelloquin R, Etard JF, Maman D, Mbala PK, Ayouba A, Peeters M, Muyembe JT, Delaporte E, Ahuka SM. *High prevalence of anti-SARS-CoV-2 antibodies after the first wave of COVID-19 in Kinshasa, Democratic Republic of the Congo: results of a cross-sectional household-based survey*. Clin Infect Dis. 2021 Jun 5:ciab515. doi: 10.1093/cid/ciab515. Epub ahead of print. PMID: 34089598; PMCID: PMC8244674.
- 3) Mariën J, Michiels J, Heyndrickx L, **Nkuba-Ndaye A**, Ceulemans A, Bartholomeeusen K, Madinga J, Mbala-Kingebeni P, Vanlerberghe V, Ahuka-Mundeke S, Wang LF, Ariën KK. *Evaluation of a surrogate virus neutralization test for high-throughput serosurveillance of SARS-CoV-2*. J Virol Methods. 2021 Nov;297:114228. doi: 10.1016/j.jviromet.2021.114228. Epub 2021 Jul 3. PMID: 34224754; PMCID: PMC8253660.
- 4) Mbala-Kingebeni P, Pratt C, Mutafali-Ruffin M, Pauthner MG, Bile F, **Nkuba-Ndaye A**, Black A, Kinganda-Lusamaki E, Faye M, Aziza A, Diagne MM, Mukadi D, White B, Hadfield J, Gangavarapu K, Bisento N, Kazadi D, Nsunda B, Akonga M, Tshiani O, Misasi J, Ploquin A, Epaso V, Sana-Paka E, N'kasar YTT, Mambu F, Edidi F, Matondo M, Bula Bula J, Diallo B, Keita M, Belizaire MRD, Fall IS, Yam A, Mulangu S, Rimion AW, Salfati E, Torkamani A, Suchard MA, Crozier I, Hensley L, Rambaut A, Faye O, Sall A, Sullivan NJ, Bedford T, Andersen KG, Wiley MR, Ahuka-Mundeke S, Muyembe Tamfum JJ. *Ebola Virus Transmission Initiated by Relapse of Systemic Ebola Virus Disease*. N Engl J Med. 2021 Apr 1;384(13):1240-1247. doi: 10.1056/NEJMoa2024670. PMID: 33789012; PMCID: PMC7888312.
- 5) Ayouba A, Thaurignac G, Morquin D, Tuailon E, Raulino R, **Nkuba A**, Lacroix A, Vidal N, Foulongne V, Le Moing V, Reynes J, Delaporte E, Peeters M. *Multiplex detection and dynamics of IgG antibodies to SARS-CoV2 and the highly pathogenic human coronaviruses SARS-CoV and MERS-CoV*. J Clin Virol. 2020 Aug;129:104521. doi: 10.1016/j.jcv.2020.104521. Epub 2020 Jun 22. PMID: 32623350; PMCID: PMC7308014.
- 6) **Nkuba-Ndaye A**, Mukadi-Bamuleka D, Bulabula-Penge J, Thaurignac G, Edidi-Atani F, Mambu-Mbika F, Danga-Yema B, Matondo-Kuamfumu M, Kinganda-Lusamaki E, Bisento N, Lumembe-Numbi R, Kabamba-Lungenyi G, Kitsa-Mutsumbirwa D, Kambale-Sivihwa N, Boillot F, Delaporte E, Mbala-Kingebeni P, Ayouba A, Peeters M, Ahuka-Mundeke S. *Added value of an anti-Ebola serology for the management of*

clinically suspect Ebola virus disease patients discharged as negative in an epidemic context. J Infect Dis. 2022 Feb 18;jiac057. doi: 10.1093/infdis/jiac057. Epub ahead of print. PMID: 35176762.

Other articles

- 1) Lacroix A, Vidal N, Keita AK, Thaurignac G, Esteban A, De Nys H, Diallo R, Toure A, Goumou S, Soumah AK, Povogui M, Koivogui J, Monemou JL, Raulino R, **Nkuba A**, Foulongne V, Delaporte E, Ayouba A, Peeters M. *Wide Diversity of Coronaviruses in Frugivorous and Insectivorous Bat Species: A Pilot Study in Guinea, West Africa.* Viruses. 2020 Aug 5;12(8):855. doi: 10.3390/v12080855. PMID: 32764506; PMCID: PMC7472279.
- 2) De Weggheleire A, **Nkuba-Ndave A**, Mbala-Kingebeni P, Mariën J, Kindombe-Luzolo E, Ilombe G, Mangala-Sonzi D, Binene-Mbuka G, De Smet B, Vogt F, Selhorst P, Matungala-Pafubel M, Nkawa F, Vulu F, Mossoko M, Pukuta-Simbu E, Kinganda-Lusamaki E, Van Bortel W, Wat'senga-Tezzo F, Makiala-Mandanda S, Ahuka-Mundeke S. A Multidisciplinary Investigation of the First Chikungunya Virus Outbreak in Matadi in the Democratic Republic of the Congo. Viruses. 2021 Oct 3;13(10):1988. doi: 10.3390/v13101988. PMID: 34696418 Free PMC article.
- 3) Selhorst P, Makiala-Mandanda S, De Smet B, Mariën J, Anthony C, Binene-Mbuka G, De Weggheleire A, Ilombe G, Kinganda-Lusamaki E, Pukuta-Simbu E, Lubula L, Mbala-Kingebeni P, **Nkuba-Ndave A**, Vogt F, Watsenga F, Van Bortel W, Vanlerberghe V, Ariën KK, Ahuka-Mundeke S. Molecular characterization of chikungunya virus during the 2019 outbreak in the Democratic Republic of the Congo. Emerg Microbes Infect. 2020 Dec;9(1):1912-1918. doi: 10.1080/22221751.2020.1810135.
- 4) Mukadi-Bamuleka D, Sanogo YO, Bulabula-Penge J, Morales-Betoulle ME, Fillon P, Woodruff P, Choi MJ, Whitesell A, Todres AM, De Weggheleire A, Legand A, Muyembe-Tamfum JJ, Formenty P, Klena JD, Montgomery JM, Ahuka-Mundeke S; RDT Working Group. Postmortem Surveillance for Ebola Virus Using OraQuick Ebola Rapid Diagnostic Tests, Eastern Democratic Republic of the Congo, 2019-2020. Emerg Infect Dis. 2022 Feb;28(2):420-424. doi: 10.3201/eid2802.210981.

Bibliographic References

- Abbasi, J. (2020). The Promise and Peril of Antibody Testing for COVID-19. *JAMA*, 323(19), 1881-1883. doi:10.1001/jama.2020.6170
- Abbasi, J. (2022). COVID-19 and the Common Cold-Preexisting Coronavirus Antibodies May Hinder SARS-CoV-2 Immunity. *JAMA*, 327(7), 609-610. doi:10.1001/jama.2022.0326
- Ackermann, M., Verleden, S. E., Kuehnel, M., *et al.* (2020). Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med*, 383(2), 120-128. doi:10.1056/NEJMoa2015432
- Adaken, C., Scott, J. T., Sharma, R., *et al.* (2021). Ebola virus antibody decay-stimulation in a high proportion of survivors. *Nature*, 590(7846), 468-472. doi:10.1038/s41586-020-03146-y
- Addetia, A., Crawford, K. H. D., Dings, A., *et al.* (2020). Neutralizing Antibodies Correlate with Protection from SARS-CoV-2 in Humans during a Fishery Vessel Outbreak with a High Attack Rate. *J Clin Microbiol*, 58(11). doi:10.1128/JCM.02107-20
- Adu-Gyamfi, E., Digman, M. A., Gratton, E., *et al.* (2012). Investigation of Ebola VP40 assembly and oligomerization in live cells using number and brightness analysis. *Biophys J*, 102(11), 2517-2525. doi:10.1016/j.bpj.2012.04.022
- Afzal, A. (2020). Molecular diagnostic technologies for COVID-19: Limitations and challenges. *J Adv Res*, 26, 149-159. doi:10.1016/j.jare.2020.08.002
- Ali, S. T., Wang, L., Lau, E. H. Y., *et al.* (2020). Serial interval of SARS-CoV-2 was shortened over time by nonpharmaceutical interventions. *Science*, 369(6507), 1106-1109. doi:10.1126/science.abc9004
- Allela, L., Boury, O., Pouillot, R., *et al.* (2005). Ebola virus antibody prevalence in dogs and human risk. *Emerg Infect Dis*, 11(3), 385-390. doi:10.3201/eid1103.040981
- Alsuliman, T., Sulaiman, R., Ismail, S., *et al.* (2020). COVID-19 paraclinical diagnostic tools: Updates and future trends. *Curr Res Transl Med*, 68(3), 83-91. doi:10.1016/j.retram.2020.06.001
- Amanna, I. J., Carlson, N. E., & Slifka, M. K. (2007). Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med*, 357(19), 1903-1915. doi:10.1056/NEJMoa066092
- Amarasinghe, G. K., Ayllon, M. A., Bao, Y., *et al.* (2019). Taxonomy of the order Mononegavirales: update 2019. *Arch Virol*, 164(7), 1967-1980. doi:10.1007/s00705-019-04247-4
- Angeloni S, Das S, De Jager W, *et al.* (2022). *xMAP® cookbook. A collection of methods and protocols for developing multiplex assays with xMAP Technology* (L. C. website Ed. 5th ed.).

- Antia, A., Ahmed, H., Handel, A., *et al.* (2018). Heterogeneity and longevity of antibody memory to viruses and vaccines. *PLoS Biol*, 16(8), e2006601. doi:10.1371/journal.pbio.2006601
- Antia, R., Regoes, R. R., Koella, J. C., *et al.* (2003). The role of evolution in the emergence of infectious diseases. *Nature*, 426(6967), 658-661. doi:10.1038/nature02104
- Apetrei, C., & Marx, P. A. (2004). Simian retroviral infections in human beings. *Lancet*, 364(9429), 137-138; author reply 139-140. doi:10.1016/S0140-6736(04)16620-8
- Aruna, A., Mbala, P., Minikulu, L., *et al.* (2019). Ebola Virus Disease Outbreak - Democratic Republic of the Congo, August 2018-November 2019. *MMWR Morb Mortal Wkly Rep*, 68(50), 1162-1165. doi:10.15585/mmwr.mm6850a3
- Arya, R., Kumari, S., Pandey, B., *et al.* (2021). Structural insights into SARS-CoV-2 proteins. *J Mol Biol*, 433(2), 166725. doi:10.1016/j.jmb.2020.11.024
- Asadi, S., Bouvier, N., Wexler, A. S., *et al.* (2020). The coronavirus pandemic and aerosols: Does COVID-19 transmit via expiratory particles? *Aerosol Sci Technol*, 0(0), 1-4. doi:10.1080/02786826.2020.1749229
- Asjo, B., & Kruse, H. (2006). Zoonoses in the Emergence of Human Viral Diseases. *Perspect Med Virol*, 16, 15-41. doi:10.1016/S0168-7069(06)16003-6
- Atherstone, C., Diederich, S., Pickering, B., *et al.* (2021). Investigation of Ebolavirus exposure in pigs presented for slaughter in Uganda. *Transbound Emerg Dis*, 68(3), 1521-1530. doi:10.1111/tbed.13822
- Audi, A., Allbrahim, M., Kaddoura, M., *et al.* (2020). Seasonality of Respiratory Viral Infections: Will COVID-19 Follow Suit? *Front Public Health*, 8, 567184. doi:10.3389/fpubh.2020.567184
- Ayouba, A., Ahuka-Mundeke, S., Butel, C., *et al.* (2019). Extensive Serological Survey of Multiple African Nonhuman Primate Species Reveals Low Prevalence of Immunoglobulin G Antibodies to 4 Ebola Virus Species. *J Infect Dis*, 220(10), 1599-1608. doi:10.1093/infdis/jiz006
- Ayouba, A., Thaurignac, G., Morquin, D., *et al.* (2020). Multiplex detection and dynamics of IgG antibodies to SARS-CoV2 and the highly pathogenic human coronaviruses SARS-CoV and MERS-CoV. *Journal of Clinical Virology*, 129(June), 104521-104521. doi:10.1016/j.jcv.2020.104521
- Ayouba, A., Toure, A., Butel, C., *et al.* (2017). Development of a Sensitive and Specific Serological Assay Based on Luminex Technology for Detection of Antibodies to Zaire Ebola Virus. *J Clin Microbiol*, 55(1), 165-176. doi:10.1128/JCM.01979-16
- Baize, S., Leroy, E. M., Georges-Courbot, M. C., *et al.* (1999). Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. *Nat Med*, 5(4), 423-426. doi:10.1038/7422

- Baize, S., Pannetier, D., Oestereich, L., *et al.* (2014). Emergence of Zaire Ebola virus disease in Guinea. *N Engl J Med*, 371(15), 1418-1425. doi:10.1056/NEJMoa1404505
- Banadyga, L., Hoenen, T., Ambroggio, X., *et al.* (2017). Ebola virus VP24 interacts with NP to facilitate nucleocapsid assembly and genome packaging. *Sci Rep*, 7(1), 7698. doi:10.1038/s41598-017-08167-8
- Baron, R. C., McCormick, J. B., & Zubeir, O. A. (1983). Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ*, 61(6), 997-1003.
- Barrette, R. W., Metwally, S. A., Rowland, J. M., *et al.* (2009). Discovery of swine as a host for the Reston ebolavirus. *Science*, 325(5937), 204-206. doi:10.1126/science.1172705
- Barry, M., Toure, A., Traore, F. A., *et al.* (2015). Clinical predictors of mortality in patients with Ebola virus disease. *Clin Infect Dis*, 60(12), 1821-1824. doi:10.1093/cid/civ202
- Baseler, L., Chertow, D. S., Johnson, K. M., *et al.* (2017). The Pathogenesis of Ebola Virus Disease. *Annu Rev Pathol*, 12, 387-418. doi:10.1146/annurev-pathol-052016-100506
- Basu, A., Zinger, T., Inglima, K., *et al.* (2020). Performance of Abbott ID Now COVID-19 Rapid Nucleic Acid Amplification Test Using Nasopharyngeal Swabs Transported in Viral Transport Media and Dry Nasal Swabs in a New York City Academic Institution. *J Clin Microbiol*, 58(8). doi:10.1128/JCM.01136-20
- Bavari, S., Bosio, C. M., Wiegand, E., *et al.* (2002). Lipid raft microdomains: a gateway for compartmentalized trafficking of Ebola and Marburg viruses. *J Exp Med*, 195(5), 593-602. doi:10.1084/jem.20011500
- Beig Parikhani, A., Bazaz, M., Bamehr, H., *et al.* (2021). The Inclusive Review on SARS-CoV-2 Biology, Epidemiology, Diagnosis, and Potential Management Options. *Curr Microbiol*, 78(4), 1099-1114. doi:10.1007/s00284-021-02396-x
- Belhadjer, Z., Meot, M., Bajolle, F., *et al.* (2020). Acute Heart Failure in Multisystem Inflammatory Syndrome in Children in the Context of Global SARS-CoV-2 Pandemic. *Circulation*, 142(5), 429-436. doi:10.1161/CIRCULATIONAHA.120.048360
- Bepouka, B., Situakibanza, H., Sangare, M., *et al.* (2022). Mortality associated with COVID-19 and hypertension in sub-Saharan Africa. A systematic review and meta-analysis. *J Clin Hypertens (Greenwich)*, 24(2), 99-105. doi:10.1111/jch.14417
- Bermejo, M., Rodriguez-Teijeiro, J. D., Illera, G., *et al.* (2006). Ebola outbreak killed 5000 gorillas. *Science*, 314(5805), 1564. doi:10.1126/science.1133105
- Bhadoria, P., Gupta, G., & Agarwal, A. (2021). Viral Pandemics in the Past Two Decades: An Overview. *Journal of Family Medicine and Primary Care*, 10(8).

- Bhatia, B., Furuyama, W., Hoenen, T., *et al.* (2021). Ebola Virus Glycoprotein Domains Associated with Protective Efficacy. *Vaccines (Basel)*, 9(6). doi:10.3390/vaccines9060630
- Blanco-Melo, D., Nilsson-Payant, B. E., Liu, W. C., *et al.* (2020). Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*, 181(5), 1036-1045 e1039. doi:10.1016/j.cell.2020.04.026
- Boczkowski, D., Nair, S. K., Snyder, D., *et al.* (1996). Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. *J Exp Med*, 184(2), 465-472. doi:10.1084/jem.184.2.465
- Boillot, F., Serrano, L., Muwonga, J., *et al.* (2016). Implementation and Operational Research: Programmatic Feasibility of Dried Blood Spots for the Virological Follow-up of Patients on Antiretroviral Treatment in Nord Kivu, Democratic Republic of the Congo. *J Acquir Immune Defic Syndr*, 71(1), e9-15. doi:10.1097/QAI.0000000000000844
- Bongaarts, J., Pelletier, F., & Gerland, G. (2009). "Global trends in AIDS mortality", Poverty, Gender, and Youth. *New York Population Council, Paper no. 16*. doi:https://doi.org/10.1007/978-90-481-9996-9_8
- Bortnick, A., Chernova, I., Quinn, W. J., 3rd, *et al.* (2012). Long-lived bone marrow plasma cells are induced early in response to T cell-independent or T cell-dependent antigens. *J Immunol*, 188(11), 5389-5396. doi:10.4049/jimmunol.1102808
- Bosio, C. M., Aman, M. J., Grogan, C., *et al.* (2003). Ebola and Marburg viruses replicate in monocyte-derived dendritic cells without inducing the production of cytokines and full maturation. *J Infect Dis*, 188(11), 1630-1638. doi:10.1086/379199
- Bourouiba, L. (2020). Turbulent Gas Clouds and Respiratory Pathogen Emissions: Potential Implications for Reducing Transmission of COVID-19. *JAMA*, 323(18), 1837-1838. doi:10.1001/jama.2020.4756
- Bowen, E. T., Lloyd, G., Harris, W. J., *et al.* (1977). Viral haemorrhagic fever in southern Sudan and northern Zaire. Preliminary studies on the aetiological agent. *Lancet*, 1(8011), 571-573. doi:10.1016/s0140-6736(77)92001-3
- Brant, A. C., Tian, W., Majerciak, V., *et al.* (2021). SARS-CoV-2: from its discovery to genome structure, transcription, and replication. *Cell Biosci*, 11(1), 136. doi:10.1186/s13578-021-00643-z
- Bray, M. (2001). The role of the Type I interferon response in the resistance of mice to filovirus infection. *J Gen Virol*, 82(Pt 6), 1365-1373. doi:10.1099/0022-1317-82-6-1365
- Bray, M., & Geisbert, T. W. (2005). Ebola virus: the role of macrophages and dendritic cells in the pathogenesis of Ebola hemorrhagic fever. *Int J Biochem Cell Biol*, 37(8), 1560-1566. doi:10.1016/j.biocel.2005.02.018

- Breman, J. G., Heymann, D. L., Lloyd, G., *et al.* (2016). Discovery and Description of Ebola Zaire Virus in 1976 and Relevance to the West African Epidemic During 2013-2016. *J Infect Dis*, 214(suppl 3), S93-S101. doi:10.1093/infdis/jiw207
- Breman, J. G., Johnson, K. M., van der Groen, G., *et al.* (1999). A search for Ebola virus in animals in the Democratic Republic of the Congo and Cameroon: ecologic, virologic, and serologic surveys, 1979-1980. Ebola Virus Study Teams. *J Infect Dis*, 179 Suppl 1, S139-147. doi:10.1086/514278
- Bres, P. (1978). [The epidemic of Ebola haemorrhagic fever in Sudan and Zaire, 1976: introductory note]. *Bull World Health Organ*, 56(2), 245.
- Broadhurst, M. J., Brooks, T. J., & Pollock, N. R. (2016). Diagnosis of Ebola Virus Disease: Past, Present, and Future. *Clin Microbiol Rev*, 29(4), 773-793. doi:10.1128/CMR.00003-16
- Brodin, P. (2021). Immune determinants of COVID-19 disease presentation and severity. *Nature Medicine*, 27(1), 28-33. doi:10.1038/s41591-020-01202-8
- Bunge, E. M., Hoet, B., Chen, L., *et al.* (2022). The changing epidemiology of human monkeypox-A potential threat? A systematic review. *PLoS Negl Trop Dis*, 16(2), e0010141. doi:10.1371/journal.pntd.0010141
- Calder, V. L., Bondeson, J., Brennan, F. M., *et al.* (2003). Antigen-specific T-cell downregulation by human dendritic cells following blockade of NF-kappaB. *Scand J Immunol*, 57(3), 261-270. doi:10.1046/j.1365-3083.2003.01228.x
- Carroll, S. A., Towner, J. S., Sealy, T. K., *et al.* (2013). Molecular evolution of viruses of the family Filoviridae based on 97 whole-genome sequences. *J Virol*, 87(5), 2608-2616. doi:10.1128/JVI.03118-12
- CDC. (2019, March 20, 2019). 1918 Pandemic (H1N1 virus). Retrieved from <https://www.cdc.gov/flu/pandemic-resources/1918-pandemic-h1n1.html#:~:text=It%20is%20estimated%20that%20about,occurring%20in%20the%20United%20States>.
- CDC. (2021a). Ebola Virus Disease Distribution Map: Cases of Ebola Virus Disease in Africa Since 1976. Retrieved from <https://www.cdc.gov/vhf/ebola/history/distribution-map.html>
- CDC. (2021b). February 2021 Democratic Republic of the Congo, North Kivu Province. Retrieved from <https://www.cdc.gov/vhf/ebola/outbreaks/drc/2021-february.html>
- CDC. (2022a). COVID-19 Treatments and Medications. Retrieved from <https://www.cdc.gov/coronavirus/2019-ncov/your-health/treatments-for-severe-illness.html>
- CDC. (2022b, February 22, 2022). History of Ebola Virus Disease (EVD) Outbreaks. Retrieved from <https://www.cdc.gov/vhf/ebola/history/chronology.html>

- CDC. (2022c, 2022-02-07). One Health Basics. Retrieved from <https://www.cdc.gov/onehealth/basics/index.html>
- CDC. (2022d, July 1, 2021). Zoonotic Diseases. Retrieved from <https://www.cdc.gov/onehealth/basics/zoonotic-diseases.html>
- Cepheid. (2022). Xpert® Ebola: Détection précise et fiable du virus Ebola Zaïre. Retrieved from <https://www.cepheid.com/fr/tests/Critical-Infectious-Diseases/Xpert-Ebola>
- Chen, K., Xiao, F., Hu, D., *et al.* (2020). SARS-CoV-2 Nucleocapsid Protein Interacts with RIG-I and Represses RIG-Mediated IFN-beta Production. *Viruses*, 13(1). doi:10.3390/v13010047
- Chen, N., Zhou, M., Dong, X., *et al.* (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*, 395(10223), 507-513. doi:10.1016/S0140-6736(20)30211-7
- Chen, S., Prettner, K., Kuhn, M., *et al.* (2021). Climate and the spread of COVID-19. *Sci Rep*, 11(1), 9042. doi:10.1038/s41598-021-87692-z
- Chen, Y., Chan, K. H., Kang, Y., *et al.* (2015). A sensitive and specific antigen detection assay for Middle East respiratory syndrome coronavirus. *Emerg Microbes Infect*, 4(4), e26. doi:10.1038/emi.2015.26
- Chen, Y., Liu, Q., & Guo, D. (2020). Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol*, 92(4), 418-423. doi:10.1002/jmv.25681
- Cheng, V. C., Wong, S. C., Chan, V. W., *et al.* (2020). Air and environmental sampling for SARS-CoV-2 around hospitalized patients with coronavirus disease 2019 (COVID-19). *Infect Control Hosp Epidemiol*, 41(11), 1258-1265. doi:10.1017/ice.2020.282
- Chertow, D. S., Kleine, C., Edwards, J. K., *et al.* (2014). Ebola virus disease in West Africa--clinical manifestations and management. *N Engl J Med*, 371(22), 2054-2057. doi:10.1056/NEJMp1413084
- Chia, P. Y., Coleman, K. K., Tan, Y. K., *et al.* (2020). Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. *Nat Commun*, 11(1), 2800. doi:10.1038/s41467-020-16670-2
- Chibwana, M. G., Jere, K. C., Kamn'gona, R., *et al.* (2020). High SARS-CoV-2 seroprevalence in health care workers but relatively low numbers of deaths in urban Malawi. *medRxiv*. doi:10.1101/2020.07.30.20164970
- Chidambaram, V., Tun, N. L., Haque, W. Z., *et al.* (2020). Factors associated with disease severity and mortality among patients with COVID-19: A systematic review and meta-analysis. *PLoS One*, 15(11), e0241541. doi:10.1371/journal.pone.0241541
- Choi, Y. K. (2021). Emerging and re-emerging fatal viral diseases. *Exp Mol Med*, 53(5), 711-712. doi:10.1038/s12276-021-00608-9

- Colavita, F., Biava, M., Castilletti, C., *et al.* (2019). Inflammatory and Humoral Immune Response during Ebola Virus Infection in Survivor and Fatal Cases Occurred in Sierra Leone during the 2014(-)2016 Outbreak in West Africa. *Viruses*, *11*(4). doi:10.3390/v11040373
- Coleman, C. M., & Frieman, M. B. (2014). Coronaviruses: important emerging human pathogens. *J Virol*, *88*(10), 5209-5212. doi:10.1128/JVI.03488-13
- Corman, V. M., Haage, V. C., Bleicker, T., *et al.* (2021). Comparison of seven commercial SARS-CoV-2 rapid point-of-care antigen tests: a single-centre laboratory evaluation study. *Lancet Microbe*, *2*(7), e311-e319. doi:10.1016/S2666-5247(21)00056-2
- Coronaviridae Study Group of the International Committee on Taxonomy of, V. (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol*, *5*(4), 536-544. doi:10.1038/s41564-020-0695-z
- Corti, D., Misasi, J., Mulangu, S., *et al.* (2016). Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody. *Science*, *351*(6279), 1339-1342. doi:10.1126/science.aad5224
- Crowe, J. E., Jr., Firestone, C. Y., & Murphy, B. R. (2001). Passively acquired antibodies suppress humoral but not cell-mediated immunity in mice immunized with live attenuated respiratory syncytial virus vaccines. *J Immunol*, *167*(7), 3910-3918. doi:10.4049/jimmunol.167.7.3910
- Cucinotta, D., & Vanelli, M. (2020). WHO Declares COVID-19 a Pandemic. *Acta Biomed*, *91*(1), 157-160. doi:10.23750/abm.v91i1.9397
- da Silva, P. G., Mesquita, J. R., de Sao Jose Nascimento, M., *et al.* (2021). Viral, host and environmental factors that favor anthroozoonotic spillover of coronaviruses: An opinionated review, focusing on SARS-CoV, MERS-CoV and SARS-CoV-2. *Sci Total Environ*, *750*, 141483. doi:10.1016/j.scitotenv.2020.141483
- da Silva, S. J. R., Silva, C., Guarines, K. M., *et al.* (2020). Clinical and Laboratory Diagnosis of SARS-CoV-2, the Virus Causing COVID-19. *ACS Infect Dis*, *6*(9), 2319-2336. doi:10.1021/acsinfecdis.0c00274
- Davis, C. W., Jackson, K. J. L., McElroy, A. K., *et al.* (2019). Longitudinal Analysis of the Human B Cell Response to Ebola Virus Infection. *Cell*, *177*(6), 1566-1582 e1517. doi:10.1016/j.cell.2019.04.036
- De Nys, H. M., Kingebeni, P. M., Keita, A. K., *et al.* (2018). Survey of Ebola Viruses in Frugivorous and Insectivorous Bats in Guinea, Cameroon, and the Democratic Republic of the Congo, 2015-2017. *Emerg Infect Dis*, *24*(12), 2228-2240. doi:10.3201/eid2412.180740
- Del Rio, C., & Malani, P. N. (2020). 2019 Novel Coronavirus-Important Information for Clinicians. *JAMA*, *323*(11), 1039-1040. doi:10.1001/jama.2020.1490

- Delamou, A., Camara, B. S., Kolie, J. P., *et al.* (2017). Profile and reintegration experience of Ebola survivors in Guinea: a cross-sectional study. *Trop Med Int Health*, 22(3), 254-260. doi:10.1111/tmi.12825
- Dennis, A., Wamil, M., Kapur, S., *et al.* (2020). Multi-organ impairment in low-risk individuals with long COVID. *medRxiv*, 2020.2010.2014.20212555-20212020.20212510.20212514.20212555. doi:10.1101/2020.10.14.20212555
- Diakou, K. I., Mitsis, T., Pierouli, K., *et al.* (2021). Ebola Virus Disease and Current Therapeutic Strategies: A Review. *Adv Exp Med Biol*, 1339, 131-137. doi:10.1007/978-3-030-78787-5_18
- Diallo, M. S. K., Ayouba, A., Keita, A. K., *et al.* (2021). Temporal evolution of the humoral antibody response after Ebola virus disease in Guinea: a 60-month observational prospective cohort study. *The Lancet Microbe*, 2(12), e676-e684. doi:10.1016/S2666-5247(21)00170-1
- Diallo, M. S. K., Rabilloud, M., Ayouba, A., *et al.* (2019). Prevalence of infection among asymptomatic and paucisymptomatic contact persons exposed to Ebola virus in Guinea: a retrospective, cross-sectional observational study. *Lancet Infect Dis*, 19(3), 308-316. doi:10.1016/S1473-3099(18)30649-2
- Diamond, M. S., & Kanneganti, T. D. (2022). Innate immunity: the first line of defense against SARS-CoV-2. *Nat Immunol*, 23(2), 165-176. doi:10.1038/s41590-021-01091-0
- Dimitriadis, G. J. (1978). Translation of rabbit globin mRNA introduced by liposomes into mouse lymphocytes. *Nature*, 274(5674), 923-924. doi:10.1038/274923a0
- Dowell, S. F., Mukunu, R., Ksiazek, T. G., *et al.* (1999). Transmission of Ebola hemorrhagic fever: a study of risk factors in family members, Kikwit, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit. *J Infect Dis*, 179 Suppl 1, S87-91. doi:10.1086/514284
- Drosten, C., Gottig, S., Schilling, S., *et al.* (2002). Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *J Clin Microbiol*, 40(7), 2323-2330. doi:10.1128/JCM.40.7.2323-2330.2002
- El Amri, H., Boukharta, M., Zakhm, F., *et al.* (2020). Emergence and Reemergence of Viral Zoonotic Diseases: Concepts and Factors of Emerging and Reemerging Globalization of Health Threats. *Emerging and Reemerging Viral Pathogens*, 619-634. doi:10.1016/B978-0-12-819400-3.00027-2
- Elie Dolgin. (2021). The tangled history of mRNA vaccines. *News Feature, Nature* 597, 318-324.
- Encyclopedia Britannica. (2022, June 30, 2022). Democratic Republic of the Congo. Retrieved from <https://www.britannica.com/place/Democratic-Republic-of-the-Congo/People>

- Epstein, M. A. (2004). Simian retroviral infections in human beings. *Lancet*, 364(9429), 138-139; author reply 139-140. doi:10.1016/S0140-6736(04)16621-X
- Falzarano, D., Krokhn, O., Van Domselaar, G., *et al.* (2007). Ebola sGP--the first viral glycoprotein shown to be C-mannosylated. *Virology*, 368(1), 83-90. doi:10.1016/j.virol.2007.06.015
- Fang, J., Pietzsch, C., Ramanathan, P., *et al.* (2018). Staufen1 Interacts with Multiple Components of the Ebola Virus Ribonucleoprotein and Enhances Viral RNA Synthesis. *mBio*, 9(5). doi:10.1128/mBio.01771-18
- Faridi, S., Niazi, S., Sadeghi, K., *et al.* (2020). A field indoor air measurement of SARS-CoV-2 in the patient rooms of the largest hospital in Iran. *Sci Total Environ*, 725, 138401. doi:10.1016/j.scitotenv.2020.138401
- Fears, A. C., Klimstra, W. B., Duprex, P., *et al.* (2020). Persistence of Severe Acute Respiratory Syndrome Coronavirus 2 in Aerosol Suspensions. *Emerg Infect Dis*, 26(9). doi:10.3201/eid2609.201806
- Feldmann, H., Jones, S., Klenk, H. D., *et al.* (2003). Ebola virus: from discovery to vaccine. *Nat Rev Immunol*, 3(8), 677-685. doi:10.1038/nri1154
- Fenollar, F., & Mediannikov, O. (2018). Emerging infectious diseases in Africa in the 21st century. *New Microbes New Infect*, 26, S10-S18. doi:10.1016/j.nmni.2018.09.004
- Fischer, K., Camara, A., Troupin, C., *et al.* (2020). Serological evidence of exposure to ebolaviruses in domestic pigs from Guinea. *Transbound Emerg Dis*, 67(2), 724-732. doi:10.1111/tbed.13391
- Fischer, K., Jabaty, J., Suluku, R., *et al.* (2018). Serological Evidence for the Circulation of Ebolaviruses in Pigs From Sierra Leone. *J Infect Dis*, 218(suppl_5), S305-S311. doi:10.1093/infdis/jiy330
- Fomsgaard, A. S., & Rosenstjerne, M. W. (2020). An alternative workflow for molecular detection of SARS-CoV-2 - escape from the NA extraction kit-shortage, Copenhagen, Denmark, March 2020. *Euro Surveill*, 25(14). doi:10.2807/1560-7917.ES.2020.25.14.2000398
- Fontanet, A., & Cauchemez, S. (2020). COVID-19 herd immunity: where are we? *Nature Reviews Immunology*, 20(10), 583-584. doi:10.1038/s41577-020-00451-5
- Forbes, K. M., Webala, P. W., Jaaskelainen, A. J., *et al.* (2019). Bombali Virus in Mops condylurus Bat, Kenya. *Emerg Infect Dis*, 25(5), 955-957. doi:10.3201/eid2505.181666
- Formenty, P., Boesch, C., Wyers, M., *et al.* (1999). Ebola virus outbreak among wild chimpanzees living in a rain forest of Cote d'Ivoire. *J Infect Dis*, 179 Suppl 1, S120-126. doi:10.1086/514296
- Foundation for Innovative New Diagnostics (FIND). (2021). SARS-CoV-2 test tracker. Retrieved from <https://www.finddx.org/covid-19/test-tracker/>

- Fryatt, A., Simms, V., Bandason, T., *et al.* (2021). Community SARS-CoV-2 seroprevalence before and after the second wave of SARS-CoV-2 infection in Harare, Zimbabwe. *EClinicalMedicine*, 41, 101172. doi:10.1016/j.eclinm.2021.101172
- Galas, A. (2014). The determinants of spread of Ebola virus disease - an evidence from the past outbreak experiences. *Folia Med Cracov*, 54(3), 17-25.
- Garraud, O. (2017). Use of convalescent plasma in Ebola virus infection. *Transfus Apher Sci*, 56(1), 31-34. doi:10.1016/j.transci.2016.12.014
- Geisbert, T. W., Hensley, L. E., Gibb, T. R., *et al.* (2000). Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. *Lab Invest*, 80(2), 171-186. doi:10.1038/labinvest.3780021
- Geisbert, T. W., Hensley, L. E., Larsen, T., *et al.* (2003). Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. *Am J Pathol*, 163(6), 2347-2370. doi:10.1016/S0002-9440(10)63591-2
- Geisbert, T. W., Jahrling, P. B., Hanes, M. A., *et al.* (1992). Association of Ebola-related Reston virus particles and antigen with tissue lesions of monkeys imported to the United States. *J Comp Pathol*, 106(2), 137-152. doi:10.1016/0021-9975(92)90043-t
- Geisbert, T. W., Strong, J. E., & Feldmann, H. (2015). Considerations in the Use of Nonhuman Primate Models of Ebola Virus and Marburg Virus Infection. *J Infect Dis*, 212 Suppl 2, S91-97. doi:10.1093/infdis/jiv284
- Geisbert, T. W., Young, H. A., Jahrling, P. B., *et al.* (2003). Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J Infect Dis*, 188(11), 1618-1629. doi:10.1086/379724
- Geisbert, T. W., Young, H. A., Jahrling, P. B., *et al.* (2003). Pathogenesis of Ebola hemorrhagic fever in primate models: evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells. *Am J Pathol*, 163(6), 2371-2382. doi:10.1016/S0002-9440(10)63592-4
- Gessain, A., Rua, R., Betsem, E., *et al.* (2013). HTLV-3/4 and simian foamy retroviruses in humans: discovery, epidemiology, cross-species transmission and molecular virology. *Virology*, 435(1), 187-199. doi:10.1016/j.virol.2012.09.035
- Ghosh, S., Saha, A., Samanta, S., *et al.* (2021). Genome structure and genetic diversity in the Ebola virus. *Curr Opin Pharmacol*, 60, 83-90. doi:10.1016/j.coph.2021.06.010
- Gibb, T. R., Norwood, D. A., Jr., Woollen, N., *et al.* (2001). Development and evaluation of a fluorogenic 5' nuclease assay to detect and differentiate between Ebola virus subtypes Zaire and Sudan. *J Clin Microbiol*, 39(11), 4125-4130. doi:10.1128/JCM.39.11.4125-4130.2001

- Gire, S. K., Goba, A., Andersen, K. G., *et al.* (2014). Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. *Science*, 345(6202), 1369-1372. doi:10.1126/science.1259657
- Glynn, J. R., Bower, H., Johnson, S., *et al.* (2017). Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-sectional study using a new non-invasive assay for antibodies to Ebola virus. *Lancet Infect Dis*, 17(6), 645-653. doi:10.1016/S1473-3099(17)30111-1
- Goldstein, T., Anthony, S. J., Gbakima, A., *et al.* (2018). The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. *Nat Microbiol*, 3(10), 1084-1089. doi:10.1038/s41564-018-0227-2
- Gong, F., Wei, H. X., Li, Q., *et al.* (2021). Evaluation and Comparison of Serological Methods for COVID-19 Diagnosis. *Front Mol Biosci*, 8, 682405. doi:10.3389/fmolb.2021.682405
- Grard, G., Biek, R., Tamfum, J. J., *et al.* (2011). Emergence of divergent Zaire ebola virus strains in Democratic Republic of the Congo in 2007 and 2008. *J Infect Dis*, 204 Suppl 3, S776-784. doi:10.1093/infdis/jir364
- Gryseels, S., Mbala-Kingebeni, P., Akonda, I., *et al.* (2020). Role of Wildlife in Emergence of Ebola Virus in Kaigbono (Likati), Democratic Republic of the Congo, 2017. *Emerg Infect Dis*, 26(9), 2205-2209. doi:10.3201/eid2609.191552
- Guan, W. J., Ni, Z. Y., Hu, Y., *et al.* (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*, 382(18), 1708-1720. doi:10.1056/NEJMoa2002032
- Gudbjartsson, D. F., Norddahl, G. L., Melsted, P., *et al.* (2020). Humoral Immune Response to SARS-CoV-2 in Iceland. *N Engl J Med*, 383(18), 1724-1734. doi:10.1056/NEJMoa2026116
- Guégan, J.-F., de Thoisy, B., Ayouba, A., *et al.* (2019). Forêts tropicales, changements d'usage des sols et risques infectieux émergents. *Santé Publique*, SI(HS1), 91-106. doi:10.3917/spub.190.0091
- Guo, Z. D., Wang, Z. Y., Zhang, S. F., *et al.* (2020). Aerosol and Surface Distribution of Severe Acute Respiratory Syndrome Coronavirus 2 in Hospital Wards, Wuhan, China, 2020. *Emerg Infect Dis*, 26(7), 1583-1591. doi:10.3201/eid2607.200885
- Habli, Z., Saleh, S., Zaraket, H., *et al.* (2020). COVID-19 in-vitro Diagnostics: State-of-the-Art and Challenges for Rapid, Scalable, and High-Accuracy Screening. *Front Bioeng Biotechnol*, 8, 605702. doi:10.3389/fbioe.2020.605702
- Haddock, E., Saturday, G., Feldmann, F., *et al.* (2021). Reston virus causes severe respiratory disease in young domestic pigs. *Proc Natl Acad Sci U S A*, 118(2). doi:10.1073/pnas.2015657118

- Hadjadj, J., Yatim, N., Barnabei, L., *et al.* (2020). Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science*, 369(6504), 718-724. doi:10.1126/science.abc6027
- Halatoko, W. A., Konu, Y. R., Gbeasor-Komlanvi, F. A., *et al.* (2020). Prevalence of SARS-CoV-2 among high-risk populations in Lomé (Togo) in 2020. *PLoS One*, 15(11), e0242124-e0242124. doi:10.1371/journal.pone.0242124
- Halfmann, P. J., Eisfeld, A. J., Watanabe, T., *et al.* (2019). Serological analysis of Ebola virus survivors and close contacts in Sierra Leone: A cross-sectional study. *PLoS Negl Trop Dis*, 13(8), e0007654. doi:10.1371/journal.pntd.0007654
- Hallal, P. C., Hartwig, F. P., Horta, B. L., *et al.* (2020). Remarkable variability in SARS-CoV-2 antibodies across Brazilian regions: Nationwide serological household survey in 27 states. *medRxiv*. doi:10.1101/2020.05.30.20117531
- Hamming, I., Timens, W., Bulthuis, M. L., *et al.* (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol*, 203(2), 631-637. doi:10.1002/path.1570
- Han, L., Zhuang, M. W., Deng, J., *et al.* (2021). SARS-CoV-2 ORF9b antagonizes type I and III interferons by targeting multiple components of the RIG-I/MDA-5-MAVS, TLR3-TRIF, and cGAS-STING signaling pathways. *J Med Virol*, 93(9), 5376-5389. doi:10.1002/jmv.27050
- Hartenian, E., Nandakumar, D., Lari, A., *et al.* (2020). The molecular virology of coronaviruses. *J Biol Chem*, 295(37), 12910-12934. doi:10.1074/jbc.REV120.013930
- Hasoksuz, M., Kilic, S., & Sarac, F. (2020). Coronaviruses and SARS-COV-2. *Turk J Med Sci*, 50(SI-1), 549-556. doi:10.3906/sag-2004-127
- Haun, B. K., Kamara, V., Dweh, A. S., *et al.* (2019). Serological evidence of Ebola virus exposure in dogs from affected communities in Liberia: A preliminary report. *PLoS Negl Trop Dis*, 13(7), e0007614. doi:10.1371/journal.pntd.0007614
- Heibati, B., Wang, W., Ryti, N. R. I., *et al.* (2021). Weather Conditions and COVID-19 Incidence in a Cold Climate: A Time-Series Study in Finland. *Frontiers in Public Health*, 8. doi:10.3389/fpubh.2020.605128
- Hensley, L. E., Young, H. A., Jahrling, P. B., *et al.* (2002). Proinflammatory response during Ebola virus infection of primate models: possible involvement of the tumor necrosis factor receptor superfamily. *Immunol Lett*, 80(3), 169-179. doi:10.1016/s0165-2478(01)00327-3
- Hicks, J., Klumpp-Thomas, C., Kalish, H., *et al.* (2021). Serologic Cross-Reactivity of SARS-CoV-2 with Endemic and Seasonal Betacoronaviruses. *J Clin Immunol*, 41(5), 906-913. doi:10.1007/s10875-021-00997-6
- Hillen, H. S., Kocic, G., Farnung, L., *et al.* (2020). Structure of replicating SARS-CoV-2 polymerase. *Nature*, 584(7819), 154-156. doi:10.1038/s41586-020-2368-8

- Hoenen, T., Groseth, A., Falzarano, D., *et al.* (2006). Ebola virus: unravelling pathogenesis to combat a deadly disease. *Trends Mol Med*, *12*(5), 206-215. doi:10.1016/j.molmed.2006.03.006
- Hoenen, T., Groseth, A., & Feldmann, H. (2019). Therapeutic strategies to target the Ebola virus life cycle. *Nat Rev Microbiol*, *17*(10), 593-606. doi:10.1038/s41579-019-0233-2
- Hoff, N. A., Bratcher, A., Kelly, J. D., *et al.* (2022). Immunogenicity of rVSVDeltaG-ZEBOV-GP Ebola vaccination in exposed and potentially exposed persons in the Democratic Republic of the Congo. *Proc Natl Acad Sci U S A*, *119*(6). doi:10.1073/pnas.2118895119
- Hoffmann, M., Kleine-Weber, H., Schroeder, S., *et al.* (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, *181*(2), 271-280 e278. doi:10.1016/j.cell.2020.02.052
- Hogan, C. A., Sahoo, M. K., Huang, C., *et al.* (2020). Five-minute point-of-care testing for SARS-CoV-2: Not there yet. *J Clin Virol*, *128*, 104410. doi:10.1016/j.jcv.2020.104410
- Hu, B., Guo, H., Zhou, P., *et al.* (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol*, *19*(3), 141-154. doi:10.1038/s41579-020-00459-7
- Huang, C., Wang, Y., Li, X., *et al.* (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, *395*(10223), 497-506. doi:10.1016/S0140-6736(20)30183-5
- Institut Pasteur. (2021). Découverte de l'ARN messager, en 1961. Retrieved from <https://www.pasteur.fr/fr/journal-recherche/actualites/decouverte-arn-messager-1961#:~:text=D%C3%A9couverte%20de%20l'ARN%20messager%2C%20en%201961>
- Isho, B., Abe, K. T., Zuo, M., *et al.* (2020). Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol*, *5*(52). doi:10.1126/sciimmunol.abe5511
- Jacob, S. T., Crozier, I., Fischer, W. A., 2nd, *et al.* (2020). Ebola virus disease. *Nat Rev Dis Primers*, *6*(1), 13. doi:10.1038/s41572-020-0147-3
- Jahrling, P. B., Geisbert, J., Swearingen, J. R., *et al.* (1996). Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses. *Arch Virol Suppl*, *11*, 135-140. doi:10.1007/978-3-7091-7482-1_12
- Jahrling, P. B., Geisbert, T. W., Dalgard, D. W., *et al.* (1990). Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet*, *335*(8688), 502-505. doi:10.1016/0140-6736(90)90737-p
- James, A. S., & Alawneh, J. I. (2020). COVID-19 Infection Diagnosis: Potential Impact of Isothermal Amplification Technology to Reduce Community Transmission of SARS-CoV-2. *Diagnostics (Basel)*, *10*(6). doi:10.3390/diagnostics10060399

- James, M. N., Edward, J.D. (editors),. (2017). Chapter 19 - Filoviridae. In N. J. MacLachlan & E. J. Dubovi (Eds.), *Fenner's Veterinary Virology (Fifth Edition)* (pp. 373-380). Boston: Academic Press.
- Jill-Léa Ramassamy. (2021). *Evaluation des risques de transmission zoonotique de virus des animaux à l'homme : étude sérologiques et épidémiologiques des virus HTLV-1 et Ebola dans les populations en contact avec des primates non-humains en Afrique Centrale.* . (PhD), Université de Paris.
- Johnson, K. M., Lange, J. V., Webb, P. A., *et al.* (1977). Isolation and partial characterisation of a new virus causing acute haemorrhagic fever in Zaire. *Lancet*, *1*(8011), 569-571. doi:10.1016/s0140-6736(77)92000-1
- Jombart, T., Jarvis, C. I., Mesfin, S., *et al.* (2020). The cost of insecurity: from flare-up to control of a major Ebola virus disease hotspot during the outbreak in the Democratic Republic of the Congo, 2019. *Euro Surveill*, *25*(2). doi:10.2807/1560-7917.ES.2020.25.2.1900735
- Jones, M. E., Schuh, A. J., Amman, B. R., *et al.* (2015). Experimental Inoculation of Egyptian Rousette Bats (*Rousettus aegyptiacus*) with Viruses of the Ebolavirus and Marburgvirus Genera. *Viruses*, *7*(7), 3420-3442. doi:10.3390/v7072779
- Juma, C. A., Mushabaa, N. K., Salam, F. A., *et al.* (2020). COVID-19: The Current Situation in the Democratic Republic of Congo. *The American Journal of Tropical Medicine and Hygiene*, *103*(6), 2168-2170. doi:10.4269/ajtmh.20-1169
- Kaneko, N., Kuo, H. H., Boucau, J., *et al.* (2020). Loss of Bcl-6-Expressing T Follicular Helper Cells and Germinal Centers in COVID-19. *Cell*, *183*(1), 143-157 e113. doi:10.1016/j.cell.2020.08.025
- Kanneganti, T. D. (2020). Intracellular innate immune receptors: Life inside the cell. *Immunol Rev*, *297*(1), 5-12. doi:10.1111/imr.12912
- Karan, L. S., Makenov, M. T., Korneev, M. G., *et al.* (2019). Bombali Virus in Mops condylurus Bats, Guinea. *Emerg Infect Dis*, *25*(9). doi:10.3201/eid2509.190581
- Kariko, K., Buckstein, M., Ni, H., *et al.* (2005). Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity*, *23*(2), 165-175. doi:10.1016/j.immuni.2005.06.008
- Karki, R., & Kanneganti, T. D. (2021). The 'cytokine storm': molecular mechanisms and therapeutic prospects. *Trends Immunol*, *42*(8), 681-705. doi:10.1016/j.it.2021.06.001
- Karki, R., Sharma, B. R., Tuladhar, S., *et al.* (2021). Synergism of TNF-alpha and IFN-gamma Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. *Cell*, *184*(1), 149-168 e117. doi:10.1016/j.cell.2020.11.025

- Keita, A. K., Butel, C., Thaurignac, G., *et al.* (2018). Serological Evidence of Ebola Virus Infection in Rural Guinea before the 2014 West African Epidemic Outbreak. *Am J Trop Med Hyg*, 99(2), 425-427. doi:10.4269/ajtmh.18-0105
- Keita, A. K., Koundouno, F. R., Faye, M., *et al.* (2021). Resurgence of Ebola virus in 2021 in Guinea suggests a new paradigm for outbreaks. *Nature*. doi:10.1038/s41586-021-03901-9
- Keita, A. K., Vidal, N., Toure, A., *et al.* (2019). A 40-Month Follow-Up of Ebola Virus Disease Survivors in Guinea (PostEbogui) Reveals Long-Term Detection of Ebola Viral Ribonucleic Acid in Semen and Breast Milk. *Open Forum Infect Dis*, 6(12), ofz482. doi:10.1093/ofid/ofz482
- Kelly, J. D., Van Ryn, C., Badio, M., *et al.* (2022). Clinical sequelae among individuals with pauci-symptomatic or asymptomatic Ebola virus infection and unrecognised Ebola virus disease in Liberia: a longitudinal cohort study. *The Lancet Infectious Diseases*, 22(8), 1163-1171. doi:10.1016/S1473-3099(22)00127-X
- Kelsall, B. L., Biron, C. A., Sharma, O., *et al.* (2002). Dendritic cells at the host-pathogen interface. *Nat Immunol*, 3(8), 699-702. doi:10.1038/ni0802-699
- Kim, P. S., Dimcheff, D. E., Siler, A., *et al.* (2022). Effect of monoclonal antibody therapy on the endogenous SARS-CoV-2 antibody response. *Clin Immunol*, 236, 108959. doi:10.1016/j.clim.2022.108959
- Kobinger, G. P., Leung, A., Neufeld, J., *et al.* (2011). Replication, pathogenicity, shedding, and transmission of Zaire ebolavirus in pigs. *J Infect Dis*, 204(2), 200-208. doi:10.1093/infdis/jir077
- Kontou, P. I., Braliou, G. G., Dimou, N. L., *et al.* (2020). Antibody Tests in Detecting SARS-CoV-2 Infection: A Meta-Analysis. *Diagnostics (Basel)*, 10(5). doi:10.3390/diagnostics10050319
- Kozak, R., He, S., Kroeker, A., *et al.* (2016). Ferrets Infected with Bundibugyo Virus or Ebola Virus Recapitulate Important Aspects of Human Filovirus Disease. *J Virol*, 90(20), 9209-9223. doi:10.1128/JVI.01033-16
- Kraemer, M. U. G., Pigott, D. M., Hill, S. C., *et al.* (2020). Dynamics of conflict during the Ebola outbreak in the Democratic Republic of the Congo 2018-2019. *BMC Med*, 18(1), 113. doi:10.1186/s12916-020-01574-1
- Krieg, P. A., & Melton, D. A. (1984). Functional messenger RNAs are produced by SP6 in vitro transcription of cloned cDNAs. *Nucleic Acids Res*, 12(18), 7057-7070. doi:10.1093/nar/12.18.7057
- Ksiazek, T. G., Rollin, P. E., Jahrling, P. B., *et al.* (1992). Enzyme immunosorbent assay for Ebola virus antigens in tissues of infected primates. *J Clin Microbiol*, 30(4), 947-950. doi:10.1128/jcm.30.4.947-950.1992

- Ksiazek, T. G., Rollin, P. E., Williams, A. J., *et al.* (1999). Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis*, *179 Suppl 1*, S177-187. doi:10.1086/514321
- Ksiazek, T. G., West, C. P., Rollin, P. E., *et al.* (1999). ELISA for the detection of antibodies to Ebola viruses. *J Infect Dis*, *179 Suppl 1*, S192-198. doi:10.1086/514313
- Kuhn, J. H. (2008). Filoviruses. A compendium of 40 years of epidemiological, clinical, and laboratory studies. *Arch Virol Suppl*, *20*, 13-360.
- Kuhn, J. H., & Bavari, S. (2017). Asymptomatic Ebola virus infections-myth or reality? *Lancet Infect Dis*, *17*(6), 570-571. doi:10.1016/S1473-3099(17)30110-X
- Kuhn, J. H., Becker, S., Ebihara, H., *et al.* (2010). Proposal for a revised taxonomy of the family Filoviridae: classification, names of taxa and viruses, and virus abbreviations. *Arch Virol*, *155*(12), 2083-2103. doi:10.1007/s00705-010-0814-x
- Kuhn, J. H., Radoshitzky, S. R., Guth, A. C., *et al.* (2006). Conserved receptor-binding domains of Lake Victoria marburgvirus and Zaire ebolavirus bind a common receptor. *J Biol Chem*, *281*(23), 15951-15958. doi:10.1074/jbc.M601796200
- Kuzmin, I. V., Niezgodna, M., Franka, R., *et al.* (2010). Marburg virus in fruit bat, Kenya. *Emerg Infect Dis*, *16*(2), 352-354. doi:10.3201/eid1602.091269
- Lacroix, A., Mbala Kingebeni, P., Ndimbo Kumugo, S. P., *et al.* (2021). Investigating the Circulation of Ebola Viruses in Bats during the Ebola Virus Disease Outbreaks in the Equateur and North Kivu Provinces of the Democratic Republic of Congo from 2018. *Pathogens*, *10*(5). doi:10.3390/pathogens10050557
- Lambert-Niclot, S., Cuffel, A., Le Pape, S., *et al.* (2020). Evaluation of a Rapid Diagnostic Assay for Detection of SARS-CoV-2 Antigen in Nasopharyngeal Swabs. *J Clin Microbiol*, *58*(8). doi:10.1128/JCM.00977-20
- Lambin, E. F., Tran, A., Vanwambeke, S. O., *et al.* (2010). Pathogenic landscapes: Interactions between land, people, disease vectors, and their animal hosts. *International Journal of Health Geographics*, *9*(1), 54. doi:10.1186/1476-072X-9-54
- Lane, H. C., & Fauci, A. S. (2021). Research in the Context of a Pandemic. *N Engl J Med*, *384*(8), 755-757. doi:10.1056/NEJMe2024638
- Lapidus, S., Liu, F., Casanovas-Massana, A., *et al.* (2021). Plasmodium infection induces cross-reactive antibodies to carbohydrate epitopes on the SARS-CoV-2 Spike protein. *medRxiv : the preprint server for health sciences*. doi:10.1101/2021.05.10.21256855
- Leisman, D. E., Ronner, L., Pinotti, R., *et al.* (2020). Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. *Lancet Respir Med*, *8*(12), 1233-1244. doi:10.1016/S2213-2600(20)30404-5

- Leligdowicz, A., Fischer, W. A., 2nd, Uyeki, T. M., *et al.* (2016). Ebola virus disease and critical illness. *Crit Care*, 20(1), 217. doi:10.1186/s13054-016-1325-2
- Leroy, E. M., Baize, S., Volchkov, V. E., *et al.* (2000). Human asymptomatic Ebola infection and strong inflammatory response. *Lancet*, 355(9222), 2210-2215. doi:10.1016/s0140-6736(00)02405-3
- Leroy, E. M., Epelboin, A., Mondonge, V., *et al.* (2009). Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector Borne Zoonotic Dis*, 9(6), 723-728. doi:10.1089/vbz.2008.0167
- Leroy, E. M., Kumulungui, B., Pourrut, X., *et al.* (2005). Fruit bats as reservoirs of Ebola virus. *Nature*, 438(7068), 575-576. doi:10.1038/438575a
- Leroy, E. M., Rouquet, P., Formenty, P., *et al.* (2004). Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science*, 303(5656), 387-390. doi:10.1126/science.1092528
- Leroy, E. M., Telfer, P., Kumulungui, B., *et al.* (2004). A serological survey of Ebola virus infection in central African nonhuman primates. *J Infect Dis*, 190(11), 1895-1899. doi:10.1086/425421
- Li, C., Yang, Y., & Ren, L. (2020). Genetic evolution analysis of 2019 novel coronavirus and coronavirus from other species. *Infect Genet Evol*, 82, 104285. doi:10.1016/j.meegid.2020.104285
- Li, J. Y., Liao, C. H., Wang, Q., *et al.* (2020). The ORF6, ORF8 and nucleocapsid proteins of SARS-CoV-2 inhibit type I interferon signaling pathway. *Virus Res*, 286, 198074. doi:10.1016/j.virusres.2020.198074
- Li, Y., Kamara, F., Zhou, G., *et al.* (2014). Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship. *PLoS Negl Trop Dis*, 8(11), e3301. doi:10.1371/journal.pntd.0003301
- Lisboa Bastos, M., Tavaziva, G., Abidi, S. K., *et al.* (2020). Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. *BMJ*, 370, m2516. doi:10.1136/bmj.m2516
- Littler, D. R., Gully, B. S., Colson, R. N., *et al.* (2020). Crystal Structure of the SARS-CoV-2 Non-structural Protein 9, Nsp9. *iScience*, 23(7), 101258. doi:10.1016/j.isci.2020.101258
- Liu, D. X., Fung, T. S., Chong, K. K., *et al.* (2014). Accessory proteins of SARS-CoV and other coronaviruses. *Antiviral Res*, 109, 97-109. doi:10.1016/j.antiviral.2014.06.013
- Liu, G., Lee, J. H., Parker, Z. M., *et al.* (2021). ISG15-dependent activation of the sensor MDA5 is antagonized by the SARS-CoV-2 papain-like protease to evade host innate immunity. *Nat Microbiol*, 6(4), 467-478. doi:10.1038/s41564-021-00884-1

- Liu, J., Trefry, J. C., Babka, A. M., *et al.* (2022). Ebola virus persistence and disease recrudescence in the brains of antibody-treated nonhuman primate survivors. *Sci Transl Med*, 14(631), eabi5229. doi:10.1126/scitranslmed.abi5229
- Lloyd-Smith, J. O. (2013). Vacated niches, competitive release and the community ecology of pathogen eradication. *Philos Trans R Soc Lond B Biol Sci*, 368(1623), 20120150. doi:10.1098/rstb.2012.0150
- Loftis, A. J., Quellie, S., Chason, K., *et al.* (2017). Validation of the Cepheid GeneXpert for Detecting Ebola Virus in Semen. *J Infect Dis*, 215(3), 344-350. doi:10.1093/infdis/jiw562
- Long, Q. X., Tang, X. J., Shi, Q. L., *et al.* (2020). Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med*, 26(8), 1200-1204. doi:10.1038/s41591-020-0965-6
- Lopez, L. A., Yang, S. J., Hauser, H., *et al.* (2010). Ebola virus glycoprotein counteracts BST-2/Tetherin restriction in a sequence-independent manner that does not require tetherin surface removal. *J Virol*, 84(14), 7243-7255. doi:10.1128/JVI.02636-09
- Lucas, C., Wong, P., Klein, J., *et al.* (2020). Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature*, 584(7821), 463-469. doi:10.1038/s41586-020-2588-y
- Lumley, S. F., O'Donnell, D., Stoesser, N. E., *et al.* (2021). Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. *N Engl J Med*, 384(6), 533-540. doi:10.1056/NEJMoa2034545
- Lumley, S. F., Wei, J., O'Donnell, D., *et al.* (2021). The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers. *Clinical Infectious Diseases*. doi:10.1093/cid/ciab004
- Lupton, H. W., Lambert, R. D., Bumgardner, D. L., *et al.* (1980). Inactivated vaccine for Ebola virus efficacious in guineapig model. *Lancet*, 2(8207), 1294-1295. doi:10.1016/s0140-6736(80)92352-1
- Lustig, Y., Keler, S., Kolodny, R., *et al.* (2020). Potential Antigenic Cross-reactivity Between Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Dengue Viruses. *Clinical Infectious Diseases*, 2(Xx Xxxx), 1-6. doi:10.1093/cid/ciaa1207
- Lustig, Y., Keler, S., Kolodny, R., *et al.* (2021). Potential Antigenic Cross-reactivity Between Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Dengue Viruses. *Clin Infect Dis*, 73(7), e2444-e2449. doi:10.1093/cid/ciaa1207
- Lyons, J. L., Tovar-y-Romo, L. B., Thakur, K. T., *et al.* (2015). Chapter 28 - Pathobiology of CNS Human Immunodeficiency Virus Infection. In M. J. Zigmond, L. P. Rowland, & J. T. Coyle (Eds.), *Neurobiology of Brain Disorders* (pp. 444-466). San Diego: Academic Press.

- MacIntyre, C. R., & Chughtai, A. A. (2016). Recurrence and reinfection--a new paradigm for the management of Ebola virus disease. *Int J Infect Dis*, *43*, 58-61. doi:10.1016/j.ijid.2015.12.011
- MacNeil, A., Farnon, E. C., Morgan, O. W., *et al.* (2011). Filovirus outbreak detection and surveillance: lessons from Bundibugyo. *J Infect Dis*, *204 Suppl 3*, S761-767. doi:10.1093/infdis/jir294
- MacNeil, A., Farnon, E. C., Wamala, J., *et al.* (2010). Proportion of deaths and clinical features in Bundibugyo Ebola virus infection, Uganda. *Emerg Infect Dis*, *16*(12), 1969-1972. doi:10.3201/eid1612.100627
- Maeda, J. M., & Nkengasong, J. N. (2021). The puzzle of the COVID-19 pandemic in Africa. *Science*, *371*(6524), 27-28. doi:10.1126/science.abf8832
- Mahanty, S., & Bray, M. (2004). Pathogenesis of filoviral haemorrhagic fevers. *Lancet Infect Dis*, *4*(8), 487-498. doi:10.1016/S1473-3099(04)01103-X
- Majiya, H., Aliyu-Paiko, M., Balogu, V. T., *et al.* (2020). Seroprevalence of COVID-19 in Niger State. *medRxiv*, 2020.2008.2004.20168112-20162020.20168108.20168104.20168112. doi:10.1101/2020.08.04.20168112
- Makiala, S., Mukadi, D., De Weggheleire, A., *et al.* (2019). Clinical Evaluation of QuickNavi(TM)-Ebola in the 2018 Outbreak of Ebola Virus Disease in the Democratic Republic of the Congo. *Viruses*, *11*(7). doi:10.3390/v11070589
- Malone, B., Urakova, N., Snijder, E. J., *et al.* (2022). Structures and functions of coronavirus replication-transcription complexes and their relevance for SARS-CoV-2 drug design. *Nat Rev Mol Cell Biol*, *23*(1), 21-39. doi:10.1038/s41580-021-00432-z
- Mandala, V. S., McKay, M. J., Shcherbakov, A. A., *et al.* (2020). Structure and Drug Binding of the SARS-CoV-2 Envelope Protein in Phospholipid Bilayers. *Res Sq*. doi:10.21203/rs.3.rs-77124/v1
- Mardian, Y., Kosasih, H., Karyana, M., *et al.* (2021). Review of Current COVID-19 Diagnostics and Opportunities for Further Development. *Front Med (Lausanne)*, *8*, 615099. doi:10.3389/fmed.2021.615099
- Mari Saez, A., Weiss, S., Nowak, K., *et al.* (2015). Investigating the zoonotic origin of the West African Ebola epidemic. *EMBO Mol Med*, *7*(1), 17-23. doi:10.15252/emmm.201404792
- Martines, R. B., Ritter, J. M., Matkovic, E., *et al.* (2020). Pathology and Pathogenesis of SARS-CoV-2 Associated with Fatal Coronavirus Disease, United States. *Emerg Infect Dis*, *26*(9), 2005-2015. doi:10.3201/eid2609.202095
- Maxmen, A. (2019). Science under fire: Ebola researchers fight to test drugs and vaccines in a war zone. *Nature*, *572*(7767), 16-17. doi:10.1038/d41586-019-02258-4

- Mbala-Kingebeni, P., Aziza, A., Di Paola, N., *et al.* (2019). Medical countermeasures during the 2018 Ebola virus disease outbreak in the North Kivu and Ituri Provinces of the Democratic Republic of the Congo: a rapid genomic assessment. *Lancet Infect Dis*, 19(6), 648-657. doi:10.1016/S1473-3099(19)30118-5
- Mbala-Kingebeni, P., Daniel Mukadi, Michel Kasereka Tosalisana, *et al.* (2022). August 2022 EVD case in DRC linked to 2018-2020 Nord Kivu EVD outbreak. Retrieved from <https://virological.org/t/august-2022-evd-case-in-drc-linked-to-2018-2020-nord-kivu-evd-outbreak/889>
- Mbala-Kingebeni, P., Pratt, C., Mutafali-Ruffin, M., *et al.* (2021). Ebola Virus Transmission Initiated by Relapse of Systemic Ebola Virus Disease. *N Engl J Med*, 384(13), 1240-1247. doi:10.1056/NEJMoa2024670
- Mbala, P., Baguelin, M., Ngay, I., *et al.* (2017). Evaluating the frequency of asymptomatic Ebola virus infection. *Philos Trans R Soc Lond B Biol Sci*, 372(1721). doi:10.1098/rstb.2016.0303
- McArthur, D. B. (2019). Emerging Infectious Diseases. *Nurs Clin North Am*, 54(2), 297-311. doi:10.1016/j.cnur.2019.02.006
- McCloskey, B., Dar, O., Zumla, A., *et al.* (2014). Emerging infectious diseases and pandemic potential: status quo and reducing risk of global spread. *Lancet Infect Dis*, 14(10), 1001-1010. doi:10.1016/S1473-3099(14)70846-1
- McCormick, J. B., Bauer, S. P., Elliott, L. H., *et al.* (1983). Biologic differences between strains of Ebola virus from Zaire and Sudan. *J Infect Dis*, 147(2), 264-267. doi:10.1093/infdis/147.2.264
- McElroy, A. K., Akondy, R. S., Davis, C. W., *et al.* (2015). Human Ebola virus infection results in substantial immune activation. *Proc Natl Acad Sci U S A*, 112(15), 4719-4724. doi:10.1073/pnas.1502619112
- McElroy, A. K., Muhlberger, E., & Munoz-Fontela, C. (2018). Immune barriers of Ebola virus infection. *Curr Opin Virol*, 28, 152-160. doi:10.1016/j.coviro.2018.01.010
- McFadden, G., Mohamed, M. R., Rahman, M. M., *et al.* (2009). Cytokine determinants of viral tropism. *Nat Rev Immunol*, 9(9), 645-655. doi:10.1038/nri2623
- Mehedi, M., Falzarano, D., Seebach, J., *et al.* (2011). A new Ebola virus nonstructural glycoprotein expressed through RNA editing. *J Virol*, 85(11), 5406-5414. doi:10.1128/JVI.02190-10
- Mehta, P., McAuley, D. F., Brown, M., *et al.* (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*, 395(10229), 1033-1034. doi:10.1016/S0140-6736(20)30628-0
- Melton, D. A., Krieg, P. A., Rebagliati, M. R., *et al.* (1984). Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a

- bacteriophage SP6 promoter. *Nucleic Acids Res*, 12(18), 7035-7056. doi:10.1093/nar/12.18.7035
- Mertens, P., De Vos, N., Martiny, D., *et al.* (2020). Development and Potential Usefulness of the COVID-19 Ag Respi-Strip Diagnostic Assay in a Pandemic Context. *Front Med (Lausanne)*, 7, 225. doi:10.3389/fmed.2020.00225
- Metcalf, C. J., Farrar, J., Cutts, F. T., *et al.* (2016). Use of serological surveys to generate key insights into the changing global landscape of infectious disease. *Lancet*, 388(10045), 728-730. doi:10.1016/S0140-6736(16)30164-7
- Mikhailov, V. V., Borisevich, I. V., Chernikova, N. K., *et al.* (1994). [The evaluation in hamadryas baboons of the possibility for the specific prevention of Ebola fever]. *Vopr Virusol*, 39(2), 82-84.
- Milleliri, J. M., Coulibaly, D., Nyobe, B., *et al.* (2021). SARS-CoV-2 infection in Ivory Coast : a serosurveillance survey among gold mine workers 1 : Groupe d ' intervention en sante publique et epidemiologie , Marseille , France , 2 : Institut National de sante publique , Abidjan , Cote d ' Ivoire ; 3 : Medicis.
- Misasi, J., Gilman, M. S., Kanekiyo, M., *et al.* (2016). Structural and molecular basis for Ebola virus neutralization by protective human antibodies. *Science*, 351(6279), 1343-1346. doi:10.1126/science.aad6117
- Moalic, V., Mercier, B., & Ferec, C. (2004). Technologie Luminex™ : principe, applications, et perspectives. *Immuno-analyse & Biologie Spécialisée*, 19(4), 181-187. doi:<https://doi.org/10.1016/j.immbio.2004.05.004>
- Mogato, M. (2009). Manila to slaughter 6,000 pigs to stop Ebola spread, Reuters. Retrieved from <https://www.reuters.com/article/idUSMAN392763>
- Moller-Tank, S., & Maury, W. (2014). Phosphatidylserine receptors: enhancers of enveloped virus entry and infection. *Virology*, 468-470, 565-580. doi:10.1016/j.virol.2014.09.009
- Moller-Tank, S., & Maury, W. (2015). Ebola virus entry: a curious and complex series of events. *PLoS Pathog*, 11(4), e1004731. doi:10.1371/journal.ppat.1004731
- Morawska, L., & Cao, J. (2020). Airborne transmission of SARS-CoV-2: The world should face the reality. *Environ Int*, 139, 105730. doi:10.1016/j.envint.2020.105730
- Morens, D. M., & Fauci, A. S. (2020). Emerging Pandemic Diseases: How We Got to COVID-19. *Cell*, 182(5), 1077-1092. doi:10.1016/j.cell.2020.08.021
- Morens, D. M., Folkers, G. K., & Fauci, A. S. (2004). The challenge of emerging and re-emerging infectious diseases. *Nature*, 430(6996), 242-249. doi:10.1038/nature02759
- Moriyama, M., Hugentobler, W. J., & Iwasaki, A. (2020). Seasonality of Respiratory Viral Infections. *Annu Rev Virol*, 7(1), 83-101. doi:10.1146/annurev-virology-012420-022445

- Morse, S. S. (1995). Factors in the emergence of infectious diseases. *Emerg Infect Dis*, 1(1), 7-15. doi:10.3201/eid0101.950102
- Mudd, P. A., Crawford, J. C., Turner, J. S., *et al.* (2020). Distinct inflammatory profiles distinguish COVID-19 from influenza with limited contributions from cytokine storm. *Sci Adv*, 6(50). doi:10.1126/sciadv.abe3024
- Mukadi-Bamuleka, D., Bulabula-Penge, J., De Weggheleire, A., *et al.* (2022). Field performance of three Ebola rapid diagnostic tests used during the 2018–20 outbreak in the eastern Democratic Republic of the Congo: a retrospective, multicentre observational study. *The Lancet Infectious Diseases*, 22(6), 891-900. doi:[https://doi.org/10.1016/S1473-3099\(21\)00675-7](https://doi.org/10.1016/S1473-3099(21)00675-7)
- Mukadi-Bamuleka, D., Sanogo, Y. O., Bulabula-Penge, J., *et al.* (2022). Postmortem Surveillance for Ebola Virus Using OraQuick Ebola Rapid Diagnostic Tests, Eastern Democratic Republic of the Congo, 2019-2020. *Emerg Infect Dis*, 28(2), 420-424. doi:10.3201/eid2802.210981
- Mukwege, D., Byabene, A. K., Akonkwa, E. M., *et al.* (2021). High SARS-CoV-2 Seroprevalence in Healthcare Workers in Bukavu, Eastern Democratic Republic of Congo. *The American Journal of Tropical Medicine and Hygiene*, 1-5. doi:10.4269/ajtmh.20-1526
- Mulangu, S., Alfonso, V. H., Hoff, N. A., *et al.* (2018). Serologic Evidence of Ebolavirus Infection in a Population With No History of Outbreaks in the Democratic Republic of the Congo. *J Infect Dis*, 217(4), 529-537. doi:10.1093/infdis/jix619
- Mulangu, S., Borchert, M., Paweska, J., *et al.* (2016). High prevalence of IgG antibodies to Ebola virus in the Efe pygmy population in the Watsa region, Democratic Republic of the Congo. *BMC Infect Dis*, 16, 263. doi:10.1186/s12879-016-1607-y
- Mulangu, S., Dodd, L. E., Davey, R. T., Jr., *et al.* (2019). A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. *N Engl J Med*, 381(24), 2293-2303. doi:10.1056/NEJMoa1910993
- Mulenga, L. B., Hines, J. Z., Fwoloshi, S., *et al.* (2021). Prevalence of SARS-CoV-2 in six districts in Zambia in July , 2020 : a cross-sectional cluster sample survey. *The Lancet Global Health*(21), 1-9. doi:10.1016/S2214-109X(21)00053-X
- Mupapa, K., Massamba, M., Kibadi, K., *et al.* (1999). Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. *J Infect Dis*, 179 Suppl 1, S18-23. doi:10.1086/514298
- Murhekar, M. V., Bhatnagar, T., Selvaraju, S., *et al.* (2021). SARS-CoV-2 antibody seroprevalence in India, August–September, 2020: findings from the second nationwide household serosurvey. *The Lancet Global Health*, 257-266. doi:10.1016/s2214-109x(20)30544-1

- Murphy, B. R., Prince, G. A., Collins, P. L., *et al.* (1991). Effect of passive antibody on the immune response of cotton rats to purified F and G glycoproteins of respiratory syncytial virus (RSV). *Vaccine*, 9(3), 185-189. doi:10.1016/0264-410x(91)90151-u
- Murray, K. A., Olivero, J., Roche, B., *et al.* (2018). Pathogeography: leveraging the biogeography of human infectious diseases for global health management. *Ecography*, 41(9), 1411-1427. doi:<https://doi.org/10.1111/ecog.03625>
- Muzembo, B. A., Ntontolo, N. P., Ngatu, N. R., *et al.* (2020). Local perspectives on Ebola during its tenth outbreak in DR Congo: A nationwide qualitative study. *PLoS One*, 15(10), e0241120. doi:10.1371/journal.pone.0241120
- Na, W., Park, N., Yeom, M., *et al.* (2015). Ebola outbreak in Western Africa 2014: what is going on with Ebola virus? *Clin Exp Vaccine Res*, 4(1), 17-22. doi:10.7774/cevr.2015.4.1.17
- Nachege, J. B., Mbala-Kingebeni, P., Otshudiema, J., *et al.* (2020). Responding to the challenge of the dual Covid-19 and ebola epidemics in the democratic republic of congo'priorities for achieving control. *American Journal of Tropical Medicine and Hygiene*, 103(2), 597-602. doi:10.4269/ajtmh.20-0642
- National Academies of Sciences, Engineering, a. M., Health and Medicine Division, *et al.* (2017). Conducting Clinical Research During an Epidemic. In E. R. Busta, M. Mancher, P. A. Cuff, K. McAdam, & G. Keusch (Eds.), *Integrating Clinical Research into Epidemic Response: The Ebola Experience*. Washington (DC).
- Navas-Martin, S. R., & Weiss, S. (2004). Coronavirus replication and pathogenesis: Implications for the recent outbreak of severe acute respiratory syndrome (SARS), and the challenge for vaccine development. *J Neurovirol*, 10(2), 75-85. doi:10.1080/13550280490280292
- Ndongo, F. A., Guichet, E., Mimbe, E. D., *et al.* (2022). Rapid Increase of Community SARS-CoV-2 Seroprevalence during Second Wave of COVID-19, Yaounde, Cameroon. *Emerg Infect Dis*, 28(6), 1233-1236. doi:10.3201/eid2806.212580
- Neal, N. (2014). Chapter 7 - Viral neuropathogenesis. In A. C. Tselis & J. Booss (Eds.), *Handbook of Clinical Neurology* (Vol. 123, pp. 175-191): Elsevier.
- Negredo, A., Palacios, G., Vazquez-Moron, S., *et al.* (2011). Discovery of an ebolavirus-like filovirus in europe. *PLoS Pathog*, 7(10), e1002304. doi:10.1371/journal.ppat.1002304
- Neil, S. J., Zang, T., & Bieniasz, P. D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature*, 451(7177), 425-430. doi:10.1038/nature06553
- NIH. (2020). Ebola Vaccines. Retrieved from [https://www.niaid.nih.gov/diseases-conditions/ebola-vaccines#:~:text=NIAID%20and%20Okairos%20\(a%20company,to%20deliver%20Ebola%20genetic%20material](https://www.niaid.nih.gov/diseases-conditions/ebola-vaccines#:~:text=NIAID%20and%20Okairos%20(a%20company,to%20deliver%20Ebola%20genetic%20material).

- NIH. (2022). Coronavirus Disease 2019: Treatment Guidelines. Retrieved from <https://www.covid19treatmentguidelines.nih.gov/about-the-guidelines/whats-new/>
- Nii-Trebi, N. I. (2017). Emerging and Neglected Infectious Diseases: Insights, Advances, and Challenges. *Biomed Res Int*, 2017, 5245021. doi:10.1155/2017/5245021
- Nkuba Ndaye, A., Hoxha, A., Madinga, J., *et al.* (2021). Challenges in interpreting SARS-CoV-2 serological results in African countries. *The Lancet. Global health*(21), 19-20. doi:10.1016/S2214-109X(21)00060-7
- Nnaji, C. A., Iwu, C. J., Ndwandwe, D. E., *et al.* (2020). Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19. *S Afr Med J*, 110(8), 759-760.
- Ohst, C., Saschenbrecker, S., Stiba, K., *et al.* (2018). Reliable Serological Testing for the Diagnosis of Emerging Infectious Diseases. *Adv Exp Med Biol*, 1062, 19-43. doi:10.1007/978-981-10-8727-1_3
- Okware, S. I., Omaswa, F. G., Zaramba, S., *et al.* (2002). An outbreak of Ebola in Uganda. *Trop Med Int Health*, 7(12), 1068-1075. doi:10.1046/j.1365-3156.2002.00944.x
- Ong, S. W. X., Tan, Y. K., Chia, P. Y., *et al.* (2020). Air, Surface Environmental, and Personal Protective Equipment Contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) From a Symptomatic Patient. *JAMA*, 323(16), 1610-1612. doi:10.1001/jama.2020.3227
- Oran, D. P., & Topol, E. J. (2021). The Proportion of SARS-CoV-2 Infections That Are Asymptomatic. *Annals of Internal Medicine*(November 2020), 1-9. doi:10.7326/m20-6976
- Ostro, M. J., Giacomoni, D., Lavelle, D., *et al.* (1978). Evidence for translation of rabbit globin mRNA after liposome-mediated insertion into a human cell line. *Nature*, 274(5674), 921-923. doi:10.1038/274921a0
- Park, W. B., Kwon, N. J., Choi, S. J., *et al.* (2020). Virus Isolation from the First Patient with SARS-CoV-2 in Korea. *J Korean Med Sci*, 35(7), e84. doi:10.3346/jkms.2020.35.e84
- Parrish, C. R., Holmes, E. C., Morens, D. M., *et al.* (2008). Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiol Mol Biol Rev*, 72(3), 457-470. doi:10.1128/MMBR.00004-08
- Pekar, J. E., Magee, A., Parker, E., *et al.* (2022). The molecular epidemiology of multiple zoonotic origins of SARS-CoV-2. *Science*, 377(6609), 960-966. doi:10.1126/science.abp8337
- Peterson, A. T. (2008). Biogeography of diseases: a framework for analysis. *Naturwissenschaften*, 95(6), 483-491. doi:10.1007/s00114-008-0352-5
- Pettitt, J., Higgs, E., Fallah, M., *et al.* (2017). Assessment and Optimization of the GeneXpert Diagnostic Platform for Detection of Ebola Virus RNA in Seminal Fluid. *J Infect Dis*, 215(4), 547-553. doi:10.1093/infdis/jiw599

- Pigott, D. M., Golding, N., Mylne, A., *et al.* (2014). Mapping the zoonotic niche of Ebola virus disease in Africa. *Elife*, 3, e04395. doi:10.7554/eLife.04395
- Pinsky, B. A., Sahoo, M. K., Sandlund, J., *et al.* (2015). Analytical Performance Characteristics of the Cepheid GeneXpert Ebola Assay for the Detection of Ebola Virus. *PLoS One*, 10(11), e0142216. doi:10.1371/journal.pone.0142216
- Ploquin, A., Zhou, Y., & Sullivan, N. J. (2018). Ebola Immunity: Gaining a Winning Position in Lightning Chess. *J Immunol*, 201(3), 833-842. doi:10.4049/jimmunol.1700827
- Pray, I. W., Gibbons-Burgener, S. N., Rosenberg, A. Z., *et al.* (2020). COVID-19 Outbreak at an Overnight Summer School Retreat - Wisconsin, July-August 2020. *MMWR Morb Mortal Wkly Rep*, 69(43), 1600-1604. doi:10.15585/mmwr.mm6943a4
- Premraj, A., Aleyas, A. G., Nautiyal, B., *et al.* (2020). Nucleic Acid and Immunological Diagnostics for SARS-CoV-2: Processes, Platforms and Pitfalls. *Diagnostics (Basel)*, 10(11). doi:10.3390/diagnostics10110866
- Pringle, C. R. (1991). The order Mononegavirales. *Arch Virol*, 117(1-2), 137-140.
- Pringle, C. R. (1997). The order Mononegavirales--current status. *Arch Virol*, 142(11), 2321-2326.
- Qiu, X., Audet, J., Wong, G., *et al.* (2013). Sustained protection against Ebola virus infection following treatment of infected nonhuman primates with ZMAb. *Sci Rep*, 3, 3365. doi:10.1038/srep03365
- Randolph, H. E., & Barreiro, L. B. (2020). Herd Immunity: Understanding COVID-19. *Immunity*, 52(5), 737-741. doi:10.1016/j.immuni.2020.04.012
- Reed, D. S., Hensley, L. E., Geisbert, J. B., *et al.* (2004). Depletion of peripheral blood T lymphocytes and NK cells during the course of ebola hemorrhagic Fever in cynomolgus macaques. *Viral Immunol*, 17(3), 390-400. doi:10.1089/vim.2004.17.390
- Richardson, E. T., Kelly, J. D., Barrie, M. B., *et al.* (2016). Minimally Symptomatic Infection in an Ebola 'Hotspot': A Cross-Sectional Serosurvey. *PLoS Negl Trop Dis*, 10(11), e0005087. doi:10.1371/journal.pntd.0005087
- Rimoin, A. W., Lu, K., Bramble, M. S., *et al.* (2018). Ebola Virus Neutralizing Antibodies Detectable in Survivors of the Yambuku, Zaire Outbreak 40 Years after Infection. *J Infect Dis*, 217(2), 223-231. doi:10.1093/infdis/jix584
- Ripperger, T. J., Uhrlaub, J. L., Watanabe, M., *et al.* (2020). Orthogonal SARS-CoV-2 Serological Assays Enable Surveillance of Low-Prevalence Communities and Reveal Durable Humoral Immunity. *Immunity*, 53(5), 925-933 e924. doi:10.1016/j.immuni.2020.10.004

- Ristanovic, E. S., Kokoskov, N. S., Crozier, I., *et al.* (2020). A Forgotten Episode of Marburg Virus Disease: Belgrade, Yugoslavia, 1967. *Microbiol Mol Biol Rev*, 84(2). doi:10.1128/MMBR.00095-19
- Rivera, A., & Messaoudi, I. (2016). Molecular mechanisms of Ebola pathogenesis. *J Leukoc Biol*, 100(5), 889-904. doi:10.1189/jlb.4RI0316-099RR
- Roarty, C., Tonry, C., McFetridge, L., *et al.* (2020). Kinetics and seroprevalence of SARS-CoV-2 antibodies in children. *The Lancet Infectious Diseases*. doi:[https://doi.org/10.1016/S1473-3099\(20\)30884-7](https://doi.org/10.1016/S1473-3099(20)30884-7)
- Robbiani, D. F., Gaebler, C., Muecksch, F., *et al.* (2020). Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature*, 584(7821), 437-442. doi:10.1038/s41586-020-2456-9
- Roels, T. H., Bloom, A. S., Buffington, J., *et al.* (1999). Ebola hemorrhagic fever, Kikwit, Democratic Republic of the Congo, 1995: risk factors for patients without a reported exposure. *J Infect Dis*, 179 Suppl 1, S92-97. doi:10.1086/514286
- Roltgen, K., Powell, A. E., Wirz, O. F., *et al.* (2020). Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. *Sci Immunol*, 5(54). doi:10.1126/sciimmunol.abe0240
- Rowe, A. K., Bertolli, J., Khan, A. S., *et al.* (1999). Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidemies a Kikwit. *J Infect Dis*, 179 Suppl 1, S28-35. doi:10.1086/514318
- Rowley, A. H. (2020). Understanding SARS-CoV-2-related multisystem inflammatory syndrome in children. *Nat Rev Immunol*, 20(8), 453-454. doi:10.1038/s41577-020-0367-5
- Rupasinghe, R., Chomel, B. B., & Martinez-Lopez, B. (2022). Climate change and zoonoses: A review of the current status, knowledge gaps, and future trends. *Acta Trop*, 226, 106225. doi:10.1016/j.actatropica.2021.106225
- Sabin, N. S., Calliope, A. S., Simpson, S. V., *et al.* (2020). Implications of human activities for (re)emerging infectious diseases, including COVID-19. *J Physiol Anthropol*, 39(1), 29. doi:10.1186/s40101-020-00239-5
- Sachs, J. D., Karim, S. S. A., Akin, L., *et al.* (2022). The Lancet Commission on lessons for the future from the COVID-19 pandemic. *The Lancet*. doi:10.1016/S0140-6736(22)01585-9
- Sagara, I., Woodford, J., Kone, M., *et al.* (2021). Rapidly increasing SARS-CoV-2 seroprevalence and limited clinical disease in three Malian communities: a prospective cohort study. *medRxiv*. doi:10.1101/2021.04.26.21256016

- Saijo, M., Niikura, M., Ikegami, T., *et al.* (2006). Laboratory diagnostic systems for Ebola and Marburg hemorrhagic fevers developed with recombinant proteins. *Clin Vaccine Immunol*, 13(4), 444-451. doi:10.1128/CVI.13.4.444-451.2006
- Sakurai, Y., Kolokoltsov, A. A., Chen, C. C., *et al.* (2015). Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment. *Science*, 347(6225), 995-998. doi:10.1126/science.1258758
- Salehi, M., Ghasemian, A., Shokouhi Mostafavi, S. K., *et al.* (2017). Seroprevalence of SARS-CoV-2 in Guilan Province, Iran, April 2020. *Iran J Pathol*, 12(2), 183-188.
- Sanchez, A., Ksiazek, T. G., Rollin, P. E., *et al.* (1999). Detection and molecular characterization of Ebola viruses causing disease in human and nonhuman primates. *J Infect Dis*, 179 Suppl 1, S164-169. doi:10.1086/514282
- Sanchez, A., Lukwiya, M., Bausch, D., *et al.* (2004). Analysis of human peripheral blood samples from fatal and nonfatal cases of Ebola (Sudan) hemorrhagic fever: cellular responses, virus load, and nitric oxide levels. *J Virol*, 78(19), 10370-10377. doi:10.1128/JVI.78.19.10370-10377.2004
- Schieffelin, J. S., Shaffer, J. G., Goba, A., *et al.* (2014). Clinical illness and outcomes in patients with Ebola in Sierra Leone. *N Engl J Med*, 371(22), 2092-2100. doi:10.1056/NEJMoa1411680
- Schnittler, H. J., & Feldmann, H. (1998). Marburg and Ebola hemorrhagic fevers: does the primary course of infection depend on the accessibility of organ-specific macrophages? *Clin Infect Dis*, 27(2), 404-406. doi:10.1086/517704
- Schubert, K., Karousis, E. D., Jomaa, A., *et al.* (2020). SARS-CoV-2 Nsp1 binds the ribosomal mRNA channel to inhibit translation. *Nat Struct Mol Biol*, 27(10), 959-966. doi:10.1038/s41594-020-0511-8
- Schultze, J. L., & Aschenbrenner, A. C. (2021). COVID-19 and the human innate immune system. *Cell*, 184(7), 1671-1692. doi:10.1016/j.cell.2021.02.029
- Scohy, A., Anantharajah, A., Bodeus, M., *et al.* (2020). Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *J Clin Virol*, 129, 104455. doi:10.1016/j.jcv.2020.104455
- Selvaraju, S., Kumar, M. S., Thangaraj, J. W. V., *et al.* (2021). Population-based serosurvey for severe acute respiratory syndrome coronavirus 2 transmission, Chennai, India. *Emerging Infectious Diseases*, 27(2), 586-589. doi:10.3201/eid2702.203938
- Semper, A. E., Broadhurst, M. J., Richards, J., *et al.* (2016). Performance of the GeneXpert Ebola Assay for Diagnosis of Ebola Virus Disease in Sierra Leone: A Field Evaluation Study. *PLoS Med*, 13(3), e1001980. doi:10.1371/journal.pmed.1001980
- Shang, J., Han, N., Chen, Z., *et al.* (2021). Compositional diversity and evolutionary pattern of coronavirus accessory proteins. *Brief Bioinform*, 22(2), 1267-1278. doi:10.1093/bib/bbaa262

- Shang, J., Ye, G., Shi, K., *et al.* (2020). Structural basis of receptor recognition by SARS-CoV-2. *Nature*, 581(7807), 221-224. doi:10.1038/s41586-020-2179-y
- Siegrist, C. A. (2003). Mechanisms by which maternal antibodies influence infant vaccine responses: review of hypotheses and definition of main determinants. *Vaccine*, 21(24), 3406-3412. doi:10.1016/s0264-410x(03)00342-6
- Siegrist, C. A., Barrios, C., Martinez, X., *et al.* (1998). Influence of maternal antibodies on vaccine responses: inhibition of antibody but not T cell responses allows successful early prime-boost strategies in mice. *Eur J Immunol*, 28(12), 4138-4148. doi:10.1002/(SICI)1521-4141(199812)28:12<4138::AID-IMMU4138>3.0.CO;2-L
- Simmons, G., Reeves, J. D., Grogan, C. C., *et al.* (2003). DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology*, 305(1), 115-123. doi:10.1006/viro.2002.1730
- Simmons, J. A., D'Souza, R. S., Ruas, M., *et al.* (2016). Ebolavirus Glycoprotein Directs Fusion through NPC1+ Endolysosomes. *J Virol*, 90(1), 605-610. doi:10.1128/JVI.01828-15
- Sinclair, J. R. (2019). Importance of a One Health approach in advancing global health security and the Sustainable Development Goals. *Rev Sci Tech*, 38(1), 145-154. doi:10.20506/rst.38.1.2949
- Singh, R. K., Dhama, K., Malik, Y. S., *et al.* (2017). Ebola virus - epidemiology, diagnosis, and control: threat to humans, lessons learnt, and preparedness plans - an update on its 40 year's journey. *Vet Q*, 37(1), 98-135. doi:10.1080/01652176.2017.1309474
- Sivanandy, P., Jun, P. H., Man, L. W., *et al.* (2022). A systematic review of Ebola virus disease outbreaks and an analysis of the efficacy and safety of newer drugs approved for the treatment of Ebola virus disease by the US Food and Drug Administration from 2016 to 2020. *J Infect Public Health*, 15(3), 285-292. doi:10.1016/j.jiph.2022.01.005
- Slanina, H., Madhugiri, R., Bylapudi, G., *et al.* (2021). Coronavirus replication-transcription complex: Vital and selective NMPylation of a conserved site in nsp9 by the NiRAN-RdRp subunit. *Proc Natl Acad Sci U S A*, 118(6). doi:10.1073/pnas.2022310118
- Slingenbergh, J. I., Gilbert, M., de Balogh, K. I., *et al.* (2004). Ecological sources of zoonotic diseases. *Rev Sci Tech*, 23(2), 467-484. doi:10.20506/rst.23.2.1492
- Smithgall, M. C., Dowlathshahi, M., Spitalnik, S. L., *et al.* (2020). Types of Assays for SARS-CoV-2 Testing: A Review. *Lab Med*, 51(5), e59-e65. doi:10.1093/labmed/lmaa039
- Steinhardt, L. C., Ige, F., Iriemenam, N. C., *et al.* (2021). Cross-Reactivity of Two SARS-CoV-2 Serological Assays in a Setting Where Malaria Is Endemic. *J Clin Microbiol*, 59(7), e0051421. doi:10.1128/JCM.00514-21
- Stringhini, S., Wisniak, A., Piumatti, G., *et al.* (2020). Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. *Lancet*, 396(10247), 313-319. doi:10.1016/S0140-6736(20)31304-0

- Sui, L., Zhao, Y., Wang, W., *et al.* (2021). SARS-CoV-2 Membrane Protein Inhibits Type I Interferon Production Through Ubiquitin-Mediated Degradation of TBK1. *Front Immunol*, 12, 662989. doi:10.3389/fimmu.2021.662989
- Sullivan, N. J., Sanchez, A., Rollin, P. E., *et al.* (2000). Development of a preventive vaccine for Ebola virus infection in primates. *Nature*, 408(6812), 605-609. doi:10.1038/35046108
- Sun, Y., Guan, X., Jia, L., *et al.* (2021). Independent and combined effects of hypertension and diabetes on clinical outcomes in patients with COVID-19: A retrospective cohort study of Huoshen Mountain Hospital and Guanggu Fangcang Shelter Hospital. *J Clin Hypertens (Greenwich)*, 23(2), 218-231. doi:10.1111/jch.14146
- Suryawanshi, R. K., Koganti, R., Agelidis, A., *et al.* (2021). Dysregulation of Cell Signaling by SARS-CoV-2. *Trends Microbiol*, 29(3), 224-237. doi:10.1016/j.tim.2020.12.007
- Susi, P. (2021). Special Issue: Virus Receptors and Viral Tropism. *Viruses*, 14(1). doi:10.3390/v14010068
- Swanepoel, R., Leman, P. A., Burt, F. J., *et al.* (1996). Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis*, 2(4), 321-325. doi:10.3201/eid0204.960407
- Swanepoel, R., Smit, S. B., Rollin, P. E., *et al.* (2007). Studies of reservoir hosts for Marburg virus. *Emerg Infect Dis*, 13(12), 1847-1851. doi:10.3201/eid1312.071115
- Tabibzadeh, A., Esghaei, M., Soltani, S., *et al.* (2021). Evolutionary study of COVID-19, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as an emerging coronavirus: Phylogenetic analysis and literature review. *Vet Med Sci*, 7(2), 559-571. doi:10.1002/vms3.394
- Takada, A., Robison, C., Goto, H., *et al.* (1997). A system for functional analysis of Ebola virus glycoprotein. *Proc Natl Acad Sci U S A*, 94(26), 14764-14769. doi:10.1073/pnas.94.26.14764
- Takahashi, K., Halfmann, P., Oyama, M., *et al.* (2013). DNA topoisomerase 1 facilitates the transcription and replication of the Ebola virus genome. *J Virol*, 87(16), 8862-8869. doi:10.1128/JVI.03544-12
- The National Academies. (2009). Microbial Evolution and Co-Adaptation. *A Tribute to the Life and Scientific Legacies of Joshua Lederberg: Workshop Summary*. Washington (DC).
- The World, B. (2019). [The Democratic Republic of the Congo: World Development Indicators].
- Thenon, N., Peyre, M., Huc, M., *et al.* (2022). COVID-19 in Africa: Underreporting, demographic effect, chaotic dynamics, and mitigation strategy impact. *PLoS Negl Trop Dis*, 16(9), e0010735. doi:10.1371/journal.pntd.0010735

- Thom, R., Tipton, T., Strecker, T., *et al.* (2021). Longitudinal antibody and T cell responses in Ebola virus disease survivors and contacts: an observational cohort study. *Lancet Infect Dis*, 21(4), 507-516. doi:10.1016/S1473-3099(20)30736-2
- Tosi, M. F. (2005). Innate immune responses to infection. *J Allergy Clin Immunol*, 116(2), 241-249; quiz 250. doi:10.1016/j.jaci.2005.05.036
- Towner, J. S., Amman, B. R., Sealy, T. K., *et al.* (2009). Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathog*, 5(7), e1000536. doi:10.1371/journal.ppat.1000536
- Towner, J. S., Pourrut, X., Albarino, C. G., *et al.* (2007). Marburg virus infection detected in a common African bat. *PLoS One*, 2(8), e764. doi:10.1371/journal.pone.0000764
- Towner, J. S., Rollin, P. E., Bausch, D. G., *et al.* (2004). Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. *J Virol*, 78(8), 4330-4341. doi:10.1128/jvi.78.8.4330-4341.2004
- Towner, J. S., Sealy, T. K., Khristova, M. L., *et al.* (2008). Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog*, 4(11), e1000212. doi:10.1371/journal.ppat.1000212
- Tshiani Mbaya, O., Mukumbayi, P., & Mulangu, S. (2021). Review: Insights on Current FDA-Approved Monoclonal Antibodies Against Ebola Virus Infection. *Front Immunol*, 12, 721328. doi:10.3389/fimmu.2021.721328
- Tvarogova, J., Madhugiri, R., Bylapudi, G., *et al.* (2019). Identification and Characterization of a Human Coronavirus 229E Nonstructural Protein 8-Associated RNA 3'-Terminal Adenylyltransferase Activity. *J Virol*, 93(12). doi:10.1128/JVI.00291-19
- United Nation High Commissioner for Refugees (2020). [Risk Communication and Community Engagement (RCCE) – COVID-19].
- Uyoga, S., Adetifa, I. M. O., Karanja, H. K., *et al.* (2021). Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Kenyan blood donors. *Science*. doi:10.1126/science.abe1916
- V'Kovski, P., Kratzel, A., Steiner, S., *et al.* (2021). Coronavirus biology and replication: implications for SARS-CoV-2. *Nat Rev Microbiol*, 19(3), 155-170. doi:10.1038/s41579-020-00468-6
- Van Caesele, P., Canadian Public Health Laboratory, N., Bailey, D., *et al.* (2020). SARS-CoV-2 (COVID-19) serology: implications for clinical practice, laboratory medicine and public health. *CMAJ*, 192(34), E973-E979. doi:10.1503/cmaj.201588
- van Doremalen, N., Bushmaker, T., Morris, D. H., *et al.* (2020). Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N Engl J Med*, 382(16), 1564-1567. doi:10.1056/NEJMc2004973

- van Griensven, J., Edwards, T., de Lamballerie, X., *et al.* (2016). Evaluation of Convalescent Plasma for Ebola Virus Disease in Guinea. *N Engl J Med*, 374(1), 33-42. doi:10.1056/NEJMoa1511812
- Vande Burgt, N. H., Kaletsky, R. L., & Bates, P. (2015). Requirements within the Ebola Viral Glycoprotein for Tetherin Antagonism. *Viruses*, 7(10), 5587-5602. doi:10.3390/v7102888
- Vivanti, A. J., Vauloup-Fellous, C., Prevot, S., *et al.* (2020). Transplacental transmission of SARS-CoV-2 infection. *Nat Commun*, 11(1), 3572. doi:10.1038/s41467-020-17436-6
- Volchkov, V. E., Becker, S., Volchkova, V. A., *et al.* (1995). GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. *Virology*, 214(2), 421-430. doi:10.1006/viro.1995.0052
- Volchkov, V. E., Feldmann, H., Volchkova, V. A., *et al.* (1998). Processing of the Ebola virus glycoprotein by the proprotein convertase furin. *Proc Natl Acad Sci U S A*, 95(10), 5762-5767. doi:10.1073/pnas.95.10.5762
- Vos, T., Lim, S. S., Abbafati, C., *et al.* (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, 396(10258), 1204-1222. doi:10.1016/S0140-6736(20)30925-9
- Walker, P. G. T., Whittaker, C., Watson, O. J., *et al.* (2020). The impact of COVID-19 and strategies for mitigation and suppression in low- And middle-income countries. *Science*, 369(6502), 413-422. doi:10.1126/science.abc0035
- Walker, P. G. T., Whittaker, C., Watson, O. J., *et al.* (2020). The impact of COVID-19 and strategies for mitigation and suppression in low- and middle-income countries. *Science*, 369(6502), 413-422. doi:10.1126/science.abc0035
- Walls, A. C., Park, Y. J., Tortorici, M. A., *et al.* (2020a). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*, 183(6), 1735. doi:10.1016/j.cell.2020.11.032
- Walls, A. C., Park, Y. J., Tortorici, M. A., *et al.* (2020b). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*, 181(2), 281-292 e286. doi:10.1016/j.cell.2020.02.058
- Wang, M. Y., Zhao, R., Gao, L. J., *et al.* (2020). SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. *Front Cell Infect Microbiol*, 10, 587269. doi:10.3389/fcimb.2020.587269
- Wang, R., Zhang, X., Irwin, D. M., *et al.* (2020). Emergence of SARS-like coronavirus poses new challenge in China. *J Infect*, 80(3), 350-371. doi:10.1016/j.jinf.2020.01.017
- Watt, A., Moukambi, F., Banadyga, L., *et al.* (2014). A novel life cycle modeling system for Ebola virus shows a genome length-dependent role of VP24 in virus infectivity. *J Virol*, 88(18), 10511-10524. doi:10.1128/JVI.01272-14

- Weingartl, H. M., Embury-Hyatt, C., Nfon, C., *et al.* (2012). Transmission of Ebola virus from pigs to non-human primates. *Sci Rep*, 2, 811. doi:10.1038/srep00811
- Weissleder, R., Lee, H., Ko, J., *et al.* (2020). COVID-19 diagnostics in context. *Sci Transl Med*, 12(546). doi:10.1126/scitranslmed.abc1931
- Wells, C. R., Pandey, A., Ndeffo Mbah, M. L., *et al.* (2019). The exacerbation of Ebola outbreaks by conflict in the Democratic Republic of the Congo. *Proc Natl Acad Sci U S A*, 116(48), 24366-24372. doi:10.1073/pnas.1913980116
- Wells, C. R., Stearns, J. K., Lutumba, P., *et al.* (2020). COVID-19 on the African continent. *Lancet Infect Dis*, 20(12), 1368-1370. doi:10.1016/S1473-3099(20)30374-1
- Wells, C. R., Stearns, J. K., Lutumba, P., *et al.* (2020). COVID-19 on the African continent. *The Lancet Infectious Diseases*, 20(12), 1368-1370. doi:10.1016/S1473-3099(20)30374-1
- Whembolua, G. L., & Tshiswaka, D. I. (2020). Public trust in the time of the coronavirus disease 2019 (Covid-19): The case of the dr congo. *Pan African Medical Journal*, 35(Supp 2), 1-2. doi:10.11604/PAMJ.SUPP.2020.35.2.22606
- WHO. (1997). Division of Emerging and Other Communicable Diseases Surveillance and Control. *Guidelines for the safe transport of infectious substances and diagnostic specimens*. Geneva: World Health Organization.
- WHO. (2019a). Ebola J&J Vaccination Campaign launched jointly by Rwanda and Democratic Republic of Congo. Retrieved from <https://www.afro.who.int/news/ebola-jj-vaccination-campaign-launched-jointly-rwanda-and-democratic-republic-congo>
- WHO. (2019b). Preliminary results on the efficacy of rVSV-ZEBOV-GP Ebola vaccine using the ring vaccination strategy in the control of an Ebola outbreak in the Democratic Republic of the Congo: an example of integration of research into epidemic response. Retrieved from <https://www.who.int/publications/m/item/preliminary-results-on-the-efficacy-of-rsvv-zebov-gp-ebola-vaccine-using-the-strategy-in-the-control-of-an-ebola-outbreak>
- WHO. (2020a). 10th Ebola outbreak in the Democratic Republic of the Congo declared over; vigilance against flare-ups and support for survivors must continue. Retrieved from <https://www.who.int/news/item/25-06-2020-10th-ebola-outbreak-in-the-democratic-republic-of-the-congo-declared-over-vigilance-against-flare-ups-and-support-for-survivors-must-continue>
- WHO. (2020b, February 12, 2020). COVID-19 Public Health Emergency of International Concern (PHEIC) Global research and innovation forum. Retrieved from [https://www.who.int/publications/m/item/covid-19-public-health-emergency-of-international-concern-\(pheic\)-global-research-and-innovation-forum#:~:text=On%2030%20January%202020%20following,of%20International%20Concern%20\(PHEIC\).](https://www.who.int/publications/m/item/covid-19-public-health-emergency-of-international-concern-(pheic)-global-research-and-innovation-forum#:~:text=On%2030%20January%202020%20following,of%20International%20Concern%20(PHEIC).)

- WHO. (2020c, January 17, 2020). Disease outbreak news - Japan. Retrieved from <https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON237>
- WHO (2020d). [Seroepidemiological investigation protocol for coronavirus 2019 (COVID-19) infection].
- WHO. (2020e, May 26, 2020). Seroepidemiological investigation protocol for coronavirus 2019 (COVID-19) infection. Retrieved from <https://www.who.int/publications/i/item/WHO-2019-nCoV-Seroepidemiology-2020.2>
- WHO. (2020f, July 9, 2020). Transmission of SARS-CoV-2: implications for infection prevention precautions. Retrieved from <https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions>
- WHO. (2020g). WHO Director-General's opening remarks at the media briefing on COVID-19-11 March 2020. Retrieved from <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020#:~:text=We%20have%20therefore%20made%20the,to%20unnecessary%20suffering%20and%20death.>
- WHO. (2021, February 21, 2021). Ebola virus disease. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease>
- WHO. (2022a). Confirmation of case of ebola virus disease in Uganda. Retrieved from <https://www.afro.who.int/news/confirmation-case-ebola-virus-disease-uganda>
- WHO. (2022b). Middle East respiratory syndrome coronavirus (MERS-CoV). Retrieved from [https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-\(mers-cov\)](https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-(mers-cov))
- WHO. (2022c). Severe Acute Respiratory Syndrome (SARS). Retrieved from https://www.who.int/health-topics/severe-acute-respiratory-syndrome#tab=tab_1
- WHO. (2022d, July 29, 2022). Tracking SARS-CoV-2 variants. Retrieved from <https://www.who.int/activities/tracking-SARS-CoV-2-variants>
- WHO. (2022e, August 2, 2022). WHO Coronavirus (COVID-19) Dashboard. Retrieved from <https://covid19.who.int/>
- WHO/International Study Team. (1978a). Ebola haemorrhagic fever in Sudan, 1976. *Bull World Health Organ*, 56(2), 247-270.
- WHO/International Study Team. (1978b). Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. *Bull World Health Organ*, 56(2), 247-270.
- WHO/International Study Team. (1978c). Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health Organ*, 56(2), 271-293.

- Wiedemann, A., Foucat, E., Hocini, H., *et al.* (2020). Long-lasting severe immune dysfunction in Ebola virus disease survivors. *Nat Commun*, *11*(1), 3730. doi:10.1038/s41467-020-17489-7
- Wiens, K. E., Nyimol Mawien, P., Rumunu, J., *et al.* (2021). Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Juba, South Sudan: a population-based study. *International Organization for Migration*, 2021.2003.2008.21253009-21252021.21253003.21253008.21253009. doi:10.1101/2021.03.08.21253009
- Wikipedia. (2022, September 20, 2022). Confinements liés à la pandémie de Covid-19 en France. Retrieved from https://fr.wikipedia.org/wiki/Confinements_li%C3%A9s_%C3%A0_la_pand%C3%A9mie_de_Covid-19_en_France#:~:text=Premier%20confinement%20national%2C%20du%2017%20mars%20au%2011%20mai%202020,-Contexte
- Williamson, E. J., Walker, A. J., Bhaskaran, K., *et al.* (2020). Factors associated with COVID-19-related death using OpenSAFELY. *Nature*, *584*(7821), 430-436. doi:10.1038/s41586-020-2521-4
- Winter, A. K., & Hegde, S. T. (2020). The important role of serology for COVID-19 control. *Lancet Infect Dis*, *20*(7), 758-759. doi:10.1016/S1473-3099(20)30322-4
- Wolfe, N. D., Daszak, P., Kilpatrick, A. M., *et al.* (2005). Bushmeat hunting, deforestation, and prediction of zoonoses emergence. *Emerg Infect Dis*, *11*(12), 1822-1827. doi:10.3201/eid1112.040789
- Wolfe, N. D., Dunavan, C. P., & Diamond, J. (2007). Origins of major human infectious diseases. *Nature*, *447*(7142), 279-283. doi:10.1038/nature05775
- Wolters, F., van de Bovenkamp, J., van den Bosch, B., *et al.* (2020). Multi-center evaluation of cepheid xpert(R) xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic. *J Clin Virol*, *128*, 104426. doi:10.1016/j.jcv.2020.104426
- Woodland, D. L., & Winslow, G. M. (2008). Immunity to emerging pathogens. *Immunol Rev*, *225*, 5-8. doi:10.1111/j.1600-065X.2008.00695.x
- Woolhouse, M. E., & Gowtage-Sequeria, S. (2005). Host range and emerging and reemerging pathogens. *Emerg Infect Dis*, *11*(12), 1842-1847. doi:10.3201/eid1112.050997
- Wrapp, D., Wang, N., Corbett, K. S., *et al.* (2020). Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation. *bioRxiv*. doi:10.1101/2020.02.11.944462
- Wratil, P. R., Schmacke, N. A., Karakoc, B., *et al.* (2021). Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses. *Cell Rep*, *37*(13), 110169. doi:10.1016/j.celrep.2021.110169

- Wu, P., Hao, X., Lau, E. H. Y., *et al.* (2020). Real-time tentative assessment of the epidemiological characteristics of novel coronavirus infections in Wuhan, China, as at 22 January 2020. *Euro Surveill*, 25(3). doi:10.2807/1560-7917.ES.2020.25.3.2000044
- Wulff, H., & Lange, J. V. (1975). Indirect immunofluorescence for the diagnosis of Lassa fever infection. *Bull World Health Organ*, 52(4-6), 429-436.
- Xu, X., Chen, P., Wang, J., *et al.* (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci*, 63(3), 457-460. doi:10.1007/s11427-020-1637-5
- Yan, R., Zhang, Y., Li, Y., *et al.* (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*, 367(6485), 1444-1448. doi:10.1126/science.abb2762
- Yong, S. E. F., Anderson, D. E., Wei, W. E., *et al.* (2020). Connecting clusters of COVID-19: an epidemiological and serological investigation. *Lancet Infect Dis*, 20(7), 809-815. doi:10.1016/S1473-3099(20)30273-5
- Yoshimoto, F. K. (2020). The Proteins of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2 or n-COV19), the Cause of COVID-19. *Protein J*, 39(3), 198-216. doi:10.1007/s10930-020-09901-4
- Yu, C. Y., Chan, K. G., Yean, C. Y., *et al.* (2021). Nucleic Acid-Based Diagnostic Tests for the Detection SARS-CoV-2: An Update. *Diagnostics (Basel)*, 11(1). doi:10.3390/diagnostics11010053
- Yu, D. S., Weng, T. H., Wu, X. X., *et al.* (2017). The lifecycle of the Ebola virus in host cells. *Oncotarget*, 8(33), 55750-55759. doi:10.18632/oncotarget.18498
- Zahouli, J. B. Z., Koudou, B. G., Muller, P., *et al.* (2017). Urbanization is a main driver for the larval ecology of Aedes mosquitoes in arbovirus-endemic settings in south-eastern Cote d'Ivoire. *PLoS Negl Trop Dis*, 11(7), e0005751. doi:10.1371/journal.pntd.0005751
- Zeng, Z., Xu, L., Xie, X. Y., *et al.* (2020). Pulmonary pathology of early-phase COVID-19 pneumonia in a patient with a benign lung lesion. *Histopathology*, 77(5), 823-831. doi:10.1111/his.14138
- Zhang, Q., Xiang, R., Huo, S., *et al.* (2021). Molecular mechanism of interaction between SARS-CoV-2 and host cells and interventional therapy. *Signal Transduct Target Ther*, 6(1), 233. doi:10.1038/s41392-021-00653-w
- Zhang, S., Xu, K., Li, C., *et al.* (2022). Long-Term Kinetics of SARS-CoV-2 Antibodies and Impact of Inactivated Vaccine on SARS-CoV-2 Antibodies Based on a COVID-19 Patients Cohort. *Front Immunol*, 13, 829665. doi:10.3389/fimmu.2022.829665
- Zhou, X., Berglund, P., Rhodes, G., *et al.* (1994). Self-replicating Semliki Forest virus RNA as recombinant vaccine. *Vaccine*, 12(16), 1510-1514. doi:10.1016/0264-410x(94)90074-4

Annexes

Annex 1

Titre complet en français

Evaluation et accompagnement des patients déclarés guéris d'une infection par le virus Ebola et de leurs contacts en République Démocratique du Congo

Titre complet en anglais

Evaluation and support of Ebola Virus Disease cured patients and their contacts in the Democratic Republic of the Congo

Titre abrégé

Les Vainqueurs d'Ebola VERSION N°3.0 DU 14/04/2020

HISTORIQUE DES VERSIONS DU PROTOCOLE

Version	Date	Motif de la modification
1.0	24/01/2020	Protocole initial
2.0	10/03/2020	Modification suite au retour du Comité d'Evaluation Ethique de l'Inserm
3.0	14/04/2020	Modification suite au retour du Comité d'Evaluation Ethique de l'Inserm Mise en cohérence des différentes partie du protocole Ajout du chapitre 9. Vigilance de la recherche

LISTE DES ABREVIATIONS

ALAT	Alanine transférase
ASAT	Aspartate amino transférase
CD4/CD8	Cluster de différenciation 4/8
CMG	Centre de méthodologie et de gestion
CNIL	Commission Nationale de l'Informatique et de Libertés
CRP	Protéine C-réactive
CS	Conseil Scientifique
CTE	Centre de traitement Ebola
CVC	Coding Variant Candidate
EBOV	Virus Ebola
Hb	Hémoglobine
HDL	Lipoprotéines de haute densité
HLA	Antigène des leucocytes humains
ICH	Conférence internationale sur l'harmonisation
ICS	Intracellular staining
IgG	Immunoglobuline G
IgM	Immunoglobuline M
INRB	Institut National de Recherche Biomédicale
Inserm	Institut national de la santé et de la recherche médicale
IP	Investigateur principal
IRD	Institut de Recherche pour le Développement
MVE	Maladie à virus Ebola
NFS	Numération formule sanguine
OMS	Organisation Mondiale de la Santé
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PNSG	Programme National de Suivi des Guéris
RDC	République Démocratique du Congo
TransVIHMI	Recherches translationnelles sur le VIH et les maladies infectieuses
UMI	Unité mixte internationale
VHB	Virus de l'hépatite B
VHC	Virus de l'hépatite C
VIH	Virus de l'immunodéficience humaine
VRI	Vaccine Research Institute

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1. ETAT DE LA QUESTION

1.1. Justification de la recherche

Le virus Ebola est l'un des plus dangereux pathogènes de l'homme et constitue un problème de santé publique émergent pour les Etats d'Afrique sub-saharienne. La maladie à virus Ebola (MVE) est apparue pour la première fois en 1976, lors de deux flambées simultanées à Nzara au Soudan et à Yambuku en République Démocratique du Congo (RDC) (1). Depuis la découverte du virus Ebola, de nombreuses flambées épidémiques (n=28) ont touché l'Afrique. Il existe 6 lignées virales distinctes dont 4 responsables d'infection chez l'Homme : Zaïre, Bundibugyo, Soudan et Tai Forest (Côte d'Ivoire). Les 2 autres étant la souche Reston (États-Unis d'Amérique et Philippines) et la souche Bombali récemment décrite chez des chauves-souris insectivores dans le district de Bombali, en Sierra Leone (2).

Ces épidémies étaient principalement localisées en Afrique Centrale, dont dix épisodes survenus en RDC. Entre 2014 et 2016, pour la première fois, trois pays de l'Afrique de l'Ouest ont été touchés par la maladie à virus Ebola avec plus de 28 000 personnes affectées et plus de 11 000 décès rapportés (3). On assiste ces dernières années à une augmentation de la fréquence des foyers épidémiques et de leur gravité. Ainsi en RDC, en 2 ans (2017 à 2018), 3 épidémies successives ont été enregistrées à Likati (Nord-Est), à l'Equateur (Nord-Ouest) et depuis plus d'un an à l'Est, dans les provinces du Nord Kivu et Ituri (4,5). L'épidémie qui sévit actuellement dans cette région (dont les premiers cas ont été notifiés en mars 2018) est la plus importante et la plus complexe jamais enregistrée en RDC. **Au 11 avril 2020, le cumul des cas est de 3456 dont 3311 confirmés. Au total, il y a 2264 décès et 1169 survivants. Aucun nouveau cas n'a été recensé depuis presque 2 mois jusqu'au 10 avril, date où un décès dû au virus Ebola a été communiqué par l'OMS. un deuxième décès a été communiqué le 13 avril.** Des cas ponctuels ont été observés dans les pays limitrophes, particulièrement en Ouganda. La ville de Goma, chef-lieu de la province du Nord Kivu, a été également touchée. L'épidémie a été difficilement maîtrisée, traduisant d'une part la réticence des populations à venir dans les centres de traitement (CTE) ; d'autre part, pour près d'un nouveau cas sur deux le contact direct à l'origine de la contamination n'était pas connu, traduisant là aussi les réticences des populations et les insuffisances des stratégies mises en place pour stopper la propagation de la maladie, notamment : le suivi des contacts et la vaccination en ceinture. Devant une telle situation, cette épidémie a été déclarée une urgence de santé publique de portée internationale par l'Organisation Mondiale de la Santé (OMS) le 17 juillet 2019.

Les épidémies précédentes d'Afrique Centrale et de l'Est dues aux sous-types Zaïre, Bundibugyo et Soudan, ont été associées à des taux de létalité très élevés, en moyenne de 65,4 % (IC 95 % [54,6 % ; 75,5 %]) [6]. Des taux comparables de mortalité ont été observés en Afrique de l'Ouest même si avec le temps on observait une amélioration de la survie liée probablement à l'amélioration de la qualité de la prise en charge et du traitement symptomatique. Pour l'épidémie actuelle, la mortalité reste importante (plus de 65%) avec un nombre important de décès communautaires enregistrés. Pour la première fois et en bénéficiant des acquis de la lutte contre l'épidémie d'Ebola en Afrique de l'Ouest, il a été possible de mettre en place un traitement spécifique soit à travers les protocoles MEURI (Monitored Emergency Use of Unregistered and Investigational Interventions) soit surtout à travers l'étude PALM randomisée évaluant plusieurs stratégies thérapeutiques basées sur des anticorps monoclonaux (ZMapp, mAb114, REGN-EB3) et un antiviral le remdésivir dont les résultats montrent une supériorité de REGN-EB3 et mAb114. La réponse à l'épidémie actuelle dans le nord-est de la République

Démocratique du Congo est également marquée par une large utilisation du vaccin anti-EBOV. A la date du 5 août 2019, 186 350 personnes ont reçu le vaccin rVSV-ZEBOV-GP dont 48 500 contacts.

L'épidémie en Afrique de l'Ouest a aussi permis d'améliorer de façon importante notre connaissance de l'infection par EBOV chez l'Homme. Ainsi l'étude des contacts des survivants en Guinée et en Sierra Leone a montré qu'entre 4 à 10% d'entre eux avaient fait une séroconversion vis-à-vis d'EBOV de façon asymptomatique ou paucisymptomatique et que ce taux variait en fonction du degré d'exposition au risque (7-9). L'impact de ces formes cliniques non diagnostiquées sur la chaîne de transmission reste à évaluer. De plus il serait important d'estimer la proportion de sujets contacts séropositifs pour EBOV parmi les contacts n'ayant pas développé la maladie, non vaccinés ou vaccinés, et les sources principales de variabilité de cette proportion.

Les études portant sur l'épidémie en Afrique de l'Ouest ont décrit aussi les conséquences de l'infection chez les survivants et cela principalement à travers deux études de référence au Liberia (PREVAIL) (10) et en Guinée (PostEbogui) (11). Avant ces études très peu de données sur les survivants étaient disponibles. Les rares études portant sur un nombre limité de survivants concernaient l'épidémie de Kikwit en République Démocratique du Congo (12,13).

Les études PREVAIL et PostEbogui ont montré, sur le plan clinique, que près de 3 survivants sur 4 présentent une plainte que l'on peut schématiquement répartir en 2 groupes, notamment : les conséquences psychologiques (dépression, anxiété...), et celles liées au virus et à la persistance d'un processus pathologique (arthralgies, atteintes oculaires, auditives, ...) sur le long terme dont les mécanismes sont mal connus. Une autre information fondamentale recueillie au cours de ces études concerne l'existence d'un potentiel réservoir viral avec la persistance du virus dans le sperme, mais aussi dans le lait maternel. Une étude en cours de publication sur le suivi des survivants de PostEbogui (pendant environ 4 ans) a montré que la présence du virus dans le sperme est près de 100% à 3 mois (après leur sortie du CTE), 75% à 6 mois puis diminue rapidement avec une dernière positivité observée 500 jours après la phase aiguë. De même dans le lait maternel, cette séropositivité a été observée jusqu'à 30 mois après la phase aiguë (14).

A la différence des études PREVAIL et PostEbogui, au cours de l'épidémie actuelle du Nord Kivu, le continuum de prise en charge est assuré tant pendant la phase aiguë que lors du suivi après la sortie du CTE, ce qui permettra d'analyser les conséquences de l'infection en fonction du tableau clinique. De plus, une grande partie de patients ont bénéficié d'un traitement antiviral ou d'anticorps monoclonaux dont il sera important d'évaluer l'impact sur les séquelles. La persistance du virus dans le sperme représente également une opportunité pour mettre en place une étude ancillaire visant à tenter de « traiter » le sperme afin d'éviter des risques d'infections secondaires. Enfin concernant la prise en charge des séquelles en fonction des pathologies observées, des protocoles spécifiques de prise en charge pourront être développés.

Un point important concerne également les enfants pour lesquels les données sur les manifestations cliniques et les séquelles restent parcellaires et discordantes.

L'étude que nous proposons permettra d'inclure les enfants guéris.

Dans l'histoire naturelle de l'infection par EBOV, au moins 3 volets concourent à l'issue de la maladie : (1) la réponse immunitaire humorale, (2) la réponse immunitaire cellulaire et (3) les facteurs génétiques de l'hôte.

Sur un plan immunologique des avancées majeures ont été obtenues, ce qui soulèvent de nouvelles questions. Sur un plan humoral, il a été possible de décrire les différentes réponses aux antigènes d'EBOV et de décrire un profil sérologique développé sur technique Luminex spécifique de l'infection (15). Cette technique a été également utilisée pour les études portant sur les contacts.

L'évolution naturelle de l'infection à Ebolavirus est caractérisée par une activation immunitaire généralisée des cellules B et T, avec une faible réponse en anticorps chez les patients décédés, une réponse immunitaire pro-inflammatoire chez les survivants et les personnes contact asymptomatiques. Aux stades ultérieurs de l'infection par le virus Ebola, une réponse immunitaire humorale persistante aux antigènes du virus Ebola a été rapportée. Une première étude réalisée par Sobarzo et ses collègues a révélé une réponse immunitaire persistante au virus Ebola Soudan 12 ans après l'épidémie à Gulu, en Ouganda (16). Un an après l'épidémie, la plupart des patients testés (18/27) présentaient des anticorps neutralisants dirigés contre les protéines NP et GP. Dix ans plus tard, 8/14 présentaient des anticorps neutralisants des deux antigènes. Une réponse immunitaire de longue durée à l'infection naturelle à Ebola par Ebola zaïre a également été évaluée chez les survivants du premier foyer EBOV signalé à Yambuku en 1976 (17). L'étude a montré que, 40 ans après l'épidémie, des anticorps anti-NP et GP de Ebolavirus zaïre pouvaient être détectés dans le plasma de 6 survivants. Certains de ces anticorps présentaient des capacités de neutralisation.

L'importance des différents isotypes d'IgG lors d'infections virales en général est controversée. Selon le type d'aide fournie par les cellules T (TH1, TH2), l'isotype d'IgG induit chez l'homme varie entre les profils IgG1 / IgG3 et IgG2 / IgG4. Au cours d'une infection naturelle à Ebolavirus, peu de données sont disponibles sur l'analyse détaillée de la réponse immunitaire aux antigènes viraux. Récemment, un typage immunologique détaillé avec les outils modernes de 4 survivants américains de l'épidémie d'Afrique de l'Ouest de 2014 a révélé que l'infection à Ebolavirus chez l'homme, dans ses phases initiales, induit une activation immunitaire substantielle, jusqu'à 50% des cellules B sont des plasmablastes et jusqu'à 30 % de cellules T activées. Une étude longitudinale des réponses des cellules B au virus EBOV chez ces quatre survivants a révélé l'induction d'anticorps IgG de longue durée spécifique du virus EBOV, avec une modification de la composition des sous-classes au fil du temps. L'IgG1 persistait, l'IgG3 diminuait rapidement et l'IgG4 apparaissait tardivement (18). Des anticorps monoclonaux spécifiques d'EBOV dérivés des cellules de ces patients ont protégé des souris expérimentalement infectées par des doses létales d'EBOV. Une autre étude récente menée dans une cohorte de 14 survivants de l'épidémie d'Afrique de l'Ouest six mois après la sortie du centre de traitement Ebola en Sierra Leone, a identifié des sous-classes polyfonctionnelles d'IgG1 et d'IgA1 chez l'ensemble des 14 patients, contrairement à d'autres sous-classes d'IgG (19). Cependant, étant donné le nombre limité de patients analysés dans ces études, on ne sait pas si ces observations peuvent être généralisées à tous les survivants du virus EBOV.

Une étude sur les pauci symptomatiques de l'épidémie d'EBOV de 1996 au Gabon a montré un biais pour une réponse IgG contre IgG1 / IgG3 chez les 6 patients étudiés, conformément au profil de cytokine pro-inflammatoire observé. Une autre étude a évalué les cellules B mémoires des survivants de l'épidémie de virus EBOV de 1995 à Kikwit, en RDC, 10 ans plus tard. Les auteurs ont produit des anticorps monoclonaux à partir de cellules B spécifiques des antigènes EBOV et deux d'entre eux (mAb100 et mAb114) ont présenté des titres

hautement neutralisants dotés d'une capacité de protection contre des doses létales d'EBOV chez les animaux(20). Les deux anticorps monoclonaux étaient également des sous-classes IgG1 ou IgG3 et le mAb114 fait partie de l'arsenal thérapeutique utilisé pour aider à contenir l'épidémie du Nord-Kivu/Ituri.

Globalement, un grand nombre de questions restent donc en suspens concernant le profil d'anticorps des survivants, en particulier le répertoire d'IgG spécifiques de ces individus et sa corrélation avec la survie et son évolution dans le temps.

L'infection à virus Ebola chez l'homme s'accompagne également de profondes altérations des réponses immunitaires innée et adaptative : blocage de la maturation et de l'activation des cellules dendritiques (DC) infectées (cellules essentielles à la présentation antigénique) (21), inhibition des réponses antivirales à interféron de type I (22) et sécrétion de grandes quantités de cytokines et chimiokines pro-inflammatoires (23). Les analyses immunologique et génétique d'individus survivants en Guinée (Cohorte PostEbogui) que nous avons réalisées montrent que ces anomalies sont encore significatives près de 2 ans après guérison, les survivants gardant un statut immunitaire qui diffère de celui des sujets sains en termes de caractérisations phénotypiques, biomarqueurs sériques et de profil génique. En effet, plusieurs marqueurs d'inflammation (IL-8, TNF- α), d'activation immunitaire (CCL5, sCD40L) et d'altération de la paroi intestinale (sCD14, LBP, iFABP, sCD163) sont significativement augmentés chez les survivants comparés à des sujets sains guinéens non infectés. De plus, la caractérisation phénotypique des sous-populations de cellules immunitaires montre une augmentation de l'expression de marqueurs d'activation à la surface des lymphocytes T CD8⁺ ainsi que de la fréquence des cellules B « exhausted », des cellules NK non-classiques et des cellules dendritiques circulantes. D'autre part, ces survivants conservent une réponse humorale IgG ainsi qu'une réponse T mémoire polyfonctionnelle spécifique du virus. Enfin, l'analyse du profil génique des survivants nous a permis de mettre en évidence un enrichissement des gènes associés à la réponse antivirale près de 2 ans après guérison.

Chez les patients survivants à l'infection, il est vraisemblable que ces anomalies persistantes puissent expliquer en partie certains signes cliniques (activation et inflammation chronique, atteinte de certains organes par un mécanisme « dysimmunitaire » comme l'existence d'uvéites ou de troubles cognitifs, douleurs chroniques).

Cette étude contribue ainsi à mieux comprendre la physiopathologie de la MVE et identifie un panel de marqueurs biologiques et génétiques qui pourraient être utilisés pour identifier une signature de "Maladie à Virus Ebola Chronique" soulevant ainsi l'importance du suivi à long terme des survivants (24).

A la suite de l'épidémie en Afrique de l'Ouest, nous ne connaissons pas l'impact des traitements initiés lors de la phase aigüe sur ces anomalies immunitaires et l'étude en RDC peut contribuer à y répondre.

Enfin, les facteurs génétiques liés à l'hôte pourraient jouer un rôle important dans la réponse au virus Ebola. Les résultats de groupes indépendants, dont le nôtre en Guinée, ont décrit à côté des survivants de cas confirmés de MVE, des contacts asymptomatiques ou pauci-symptomatiques avec des anticorps spécifiques des antigènes du virus Ebola ainsi que des contacts ne portant pas d'anticorps et pouvant être considérés comme non infectés. Ainsi, certaines personnes fortement exposées au virus ne sont pas infectées et certaines personnes infectées par le virus ne développent pas de maladie clinique (11). Le rôle majeur des facteurs

généétiques humains dans la réponse aux agents infectieux est maintenant bien établi (25). C'est notamment le cas des infections virales concernant les deux principales étapes du processus allant de 1) l'exposition à l'infection et 2) de l'infection à l'apparition d'une maladie clinique.

La variabilité observée au cours de l'infection par EBOV rend également très probable l'existence de facteurs génétiques humains influant sur la réponse au virus. Les origines génétiques de l'hôte ont été impliquées dans la pathogenèse d'Ebola dans des modèles animaux (26), montrant une variabilité considérable des souches des ours allant de la résistance totale à l'EBOV à une létalité de 100% (27). On sait également que de nombreuses molécules hôtes sont impliquées dans l'entrée cellulaire de filovirus, y compris EBOV (28), en particulier Niemann-Pick C1 (NPC1) (29, 30). Des études chez l'animal ont montré que NPC1 influençait fortement les infections à filovirus (31, 32) et des études cellulaires in vitro récentes suggéraient que les SNP (single- nucleotide polymorphism) de NPC1 humains pouvaient également affecter la susceptibilité cellulaire aux filovirus (33). D'autres molécules et voies ont été impliquées dans la réponse à l'infection par EBOV, telles que les canaux calciques endosomaux à deux pores (34), l'ubiquitine ligase RBBP6 (35) et les protéines associées à l'autophagie (36). **Toutefois, les variants génétiques humains pouvant être impliqués dans la réponse au virus Ebola n'ont pas encore été étudiés à l'aide de cohortes bien définies.**

Au plan politique et social, l'épidémie d'Ebola en RDC s'inscrit dans la durée et ce malgré le déploiement d'équipes aguerries à la lutte contre l'épidémie, l'expérience de la RDC en la matière et les énormes progrès dans la lutte contre les fièvres hémorragiques virales tant au niveau thérapeutique que préventif. Les réponses technologiques (qu'il s'agisse du séquençage en temps réel des souches Ebola des nouveaux cas pour retracer les chaînes de contamination par analyse phylogénétique (4), de la vaccination ou de l'utilisation d'unités individuelles d'isolation) montrent les limites de leur efficacité.

Aussi l'organisation d'une réponse à une épidémie, quelle qu'elle soit :

- Ne peut faire l'impasse de la prise en compte du contexte social et politique dans lequel elle s'inscrit ;
- Ne peut ignorer que la réponse à l'épidémie est de nature interactionnelle : les termes de l'interaction entre les populations et les acteurs de la riposte ne sont pas donnés mais se construisent dans le temps ;
- Repose sur la confiance. Celle-ci est nécessaire à l'établissement d'une interaction apaisée, elle ne se décrète pas mais s'acquiert en grande partie par un apprivoisement mutuel qui se construit dans l'expérience et dans la durée

Or ce contexte reste la part aveugle dans la compréhension des transmissions de l'épidémie décrits dans les arbres phylogénétiques (4), dans les déclarations de rupture de suivi de contact ou dans l'impossibilité de ce dernier. Un abîme sépare le contexte de la contagion tel que décrit dans les arbres phylogénétiques. Les contagions sont décrites, mais souvent avec plusieurs gaps. Mais dans tous les cas, ces cartographies de la contagion sont peu prolixes sur le contexte interactionnel, ou sur les logiques du care qui ont présidé à cette contagion. L'enjeu ici consiste à saisir la tension entre contexte représentationnel (qui sous-tend les représentations phylogénétiques) et le contexte interactionnel (qui sous-tend les logiques d'actions des acteurs et détermine les routes de la contagion). Ce contexte n'est pas figé et les termes de l'interaction sont en permanence redéfinis en fonction d'enjeux politiques et sociaux qu'il faut saisir (37).

Partant de ce constat, nous envisageons de développer en parallèle des recherches menées sur les conditions physiologiques de circulation du virus et de la contagion, une étude rétrospective sur cette circulation afin d'identifier les logiques pratiques de la circulation virale et les logiques de la soustraction au suivi.

On étudiera l'impact de ce contexte sur les logiques de la contagion comme sur les conditions du suivi de contact. Partant des cas de survivants, nous reconstituerons a posteriori les moments de contagion, les logiques pratiques déployées par les acteurs (soit pour se plier aux logiques sanitaires en temps épidémique soit pour s'en soustraire).

1.2. Retombées attendues

Le but de cette étude est d'apporter une meilleure connaissance globale de l'infection par le virus Ebola et de ses conséquences cliniques, virologiques et immunologiques, des patients survivants et de leurs contacts. Ce projet permettra de renforcer le suivi pluridisciplinaire des patients après une phase aiguë de la maladie à virus Ebola en République Démocratique du Congo. Les résultats auront donc un impact direct pour la prise en charge clinique de cette population et pour la prévention des contaminations secondaires éventuelles.

Cette étude structurante contribuera au renforcement des capacités de recherche de l'INRB en particulier dans le domaine de la recherche clinique ainsi qu'au renforcement des infrastructures au niveau des sites participants à l'étude (financement complémentaire à ce protocole dans le cadre de la réponse Française à l'épidémie d'Ebola).

2. OBJECTIFS

Etudier les conséquences cliniques, immunologiques et virologiques de la maladie et de son traitement chez les survivants à la MVE.

Etudier les caractéristiques cliniques, sérologiques, virologiques, immunologique des personnes contacts des patients guéris de la cohorte « Les Vainqueurs d'Ebola ».

Explorer et caractériser chez les contacts des patients guéris de la cohorte « Les Vainqueurs d'Ebola » d'éventuelles infections peu ou pauci symptomatiques à travers des enquêtes sérologiques, ainsi que l'expérience du risque et des mesures de *contact tracing* et de vaccination.

Etudier les facteurs génétiques pouvant expliquer les différentes évolutions possibles de l'infection à EBOV en comparant des survivants à la MVE, des sujets contacts non infectés et des sujets contacts infectés.

3. CRITERES DE JUGEMENT

Chez les survivants :

- Description de la population : Fréquence des co-infections (VIH, VHB, VHC), comorbidités et fréquence et sévérité des maladies infectieuses usuelles,
- Conséquences cliniques : présence de séquelles et leur évolution au cours du temps selon les caractéristiques cliniques et le type de traitement reçu lors de l'hospitalisation,
- Conséquences immunologiques humorales : proportion des sous-classes d'IgG dirigées contre la nucléoprotéine, la VP40 et la glycoprotéine des isolats Mayinga1976 et Kissidougou-Makona2014 et évolution

de la concentration du sous-type d'IgG dominant chez chacun des survivants par analyse sérologique basée sur la technologie Luminex®,

- Conséquences virologiques : évolution de la présence du virus EBOV dans le sang, les urines, les fèces, la salive, les larmes, le lait maternel (femmes allaitantes), les sécrétions génitales (femmes âgées de plus de 15 ans) et le sperme (homme âgés de 15 ans et plus) évalué par PCR.

Chez les contacts des survivants à la MVE :

- Description du risque d'exposition au virus Ebola : description de l'exposition au cas index confirmé (durée et répétition d'exposition, protection éventuelle utilisée, état de contagiosité du cas index confirmé dont l'état clinique et la virémie), nombre de personnes atteintes de MVE, vivantes ou décédées, avec qui le sujet contact a été en présence, statut vaccinal contre la MVE.
- Fréquence d'infections asymptomatiques ou pauci-symptomatiques : description de l'état clinique depuis l'exposition au cas index par un entretien avec le contact, dosage des anticorps anti-glycoprotéine, anti-nucléoprotéine et anti-VP40 par les méthodes ELISA et Luminex®, présence du virus EBOV dans le sang, les urines, les larmes, les selles, la salive, le lait maternel (femmes allaitantes), les sécrétions cervico-vaginales (femmes âgées de plus de 15 ans) et le sperme (hommes âgés de 15 ans et plus) évalué par PCR chez les contacts présentant des anticorps anti-protéine d'EBOV.

Dans une sous-population de survivants (120) et de contacts non infectés (80 : 40 vaccinés + 40 non vaccinés) adultes :

- Etude immunologique cellulaire : Analyse du statut immunitaire des survivants : i) Caractérisation phénotypique et statut d'activation des différentes populations cellulaires au sein des PBMC (cellules T, cellules B, cellules NK, DC, etc...) par cytométrie en flux ii) Dosages sériques de différents analytes impliqués dans les réponses immunes par technologie Luminex iii) Evaluation des réponses T EBOV-spécifiques après stimulation des cellules en cytométrie en flux iv) Analyse du profil d'expression de gènes dans le sang total par technologie Illumina. Dans un sous-groupe de patients pour lequel du matériel biologique serait encore disponible après les analyses i) à iv), il sera également possible de faire une analyse « single cell » (10X Genomics) afin d'évaluer l'hétérogénéité génique d'une cellule au sein d'une population cellulaire définie dans le but de caractériser plus finement une population d'intérêt.

Dans une sous-population adulte de survivants (50), de contacts non infectés (50) et de contacts infectés (50) : Etude génétique des différents sous-groupes pouvant expliquer les différentes évolutions d'EBOV par la technique Whole Exome Sequencing pour rechercher les variants génétiques impliqués dans la réponse à l'infection par EBOV.

4. CONCEPTION DE LA RECHERCHE

4.1. Type de recherche

Il s'agit d'une étude de cohorte ouverte, multicentrique, multidisciplinaire, observationnelle de type descriptif et analytique chez des adultes et des enfants déclarés guéris après une infection à virus Ebola à la sortie de leur centre de traitement.

A partir de cette étude de cohorte, différentes études ancillaires immunologiques, génétiques, thérapeutiques seront proposées à la fois chez les guéris mais aussi leurs contacts.

4.2. Calendrier de l'étude

4.2.1. Population des survivants

La durée initiale prévue de la recherche est de 18 mois. Pour cette première phase, la durée des inclusions sera de 6 mois avec un suivi de 12 mois pour le dernier patient inclus. Les patients seront suivis jusqu'au 12^{ème} mois de suivi du dernier participant inclus ; les premiers patients seront donc suivis sur 18 mois. Lors de ce suivi, 120 survivants seront inclus dans l'étude d'immunologie cellulaire et 50 survivants dans l'étude génétique. Un suivi prolongé pourra être envisagé en fonction des ressources disponibles et sur proposition du conseil scientifique de l'étude.

4.2.2. Population des contacts

La durée des inclusions sera de 6 mois, identique à celle des survivants. Une seule visite d'inclusion (screening) est prévue où toutes les informations nécessaires à l'étude seront récoltées. Les 100 échantillons des 100 contacts de l'étude génétique seront également prélevés lors de cette visite d'inclusion. En cas de sérologie EBOV positive une seconde visite sera effectuée dans les 2 semaines suivant la première visite afin d'informer le contact et vérifier l'absence de virus dans les liquides biologiques. Pour les 80 contacts inclus dans l'étude d'immunologie cellulaire, une deuxième visite sera également programmée dans les deux semaines suivant la première visite.

5. POPULATION ETUDIEE

5.1. Description

La population étudiée est constituée des personnes guéries (« les survivants ») à leur sortie du centre de traitement après une phase aiguë d'infection à virus Ebola confirmée (deux PCR négatives à au moins 24 heures d'intervalle) ainsi que de leurs contacts.

5.2. Critères d'inclusion

5.2.1. Population des survivants

Dans la population des survivants, sont éligibles les personnes répondant aux critères suivants :

- Age \geq 5 ans,
- Adultes et enfants après un épisode aigu déclaré guéri de maladie à virus Ebola confirmé biologiquement (il faut deux PCR négatives à au moins 24 heures d'intervalle pour qu'un patient ne présentant pas de symptôme clinique puisse sortir du centre de traitement Ebola),
- Pour les mineurs, consentement éclairé signé par au moins un des deux parents ou le tuteur légal autorisant la participation de l'enfant à l'étude,
- Participant volontaire ayant signé le consentement éclairé pour les adultes.

Pour être inclus dans l'étude d'immunologie cellulaire, les 120 survivants devront avoir 18 ans ou plus, accepter de participer à cette étude et avoir une PCR du sang négative pour EBOV. Soixante survivants devront avoir reçu et 60 devront ne pas avoir reçu de traitement antiviral pendant la phase aigüe de l'infection.

5.2.2. Population des contacts

Dans la population des contacts, sont éligibles les personnes répondant aux critères suivants :

- Age \geq 5 ans,
- Etre une personne contact d'un patient guéri (i.e. malade ayant présenté une MVE prouvée, et avec deux PCR négatives à au moins 24 heures d'intervalle) inclus dans la cohorte des Vainqueurs d'Ebola. A partir des recommandations OMS et des Centers for Disease Control and Prevention pour l'identification et la traçabilité des sujets contact, une personne contact est définie comme une personne ayant côtoyé le cas index au cours de sa MVE dans sa résidence, c'est à dire ayant le même lieu de vie que lui (6-7),
- Ne pas avoir été diagnostiqué malade de la MVE,
- Accord de participation :
 - Pour les participants majeurs (\geq 18 ans, émancipés ou mariés) : consentement éclairé destiné au participant majeur signé,
 - Pour les mineurs \geq 5 ans : consentement éclairé destiné au(x) parent(s)/tuteur légal signé par au moins un des deux parents ou le tuteur légal et formulaire d'assentiment destiné au participant complété.

Pour être inclus dans l'étude d'immunologie cellulaire, les 80 contacts prévus devront :

- être âgés de 18 ans et plus,
- accepter de participer à cette étude,
- avoir une PCR du sang négative pour EBOV,.
-

Parmi les contacts inclus dans l'étude d'immunologie cellulaire, quarante devront avoir reçu un vaccin contre le virus Ebola et 40 devront ne pas en avoir reçu.

5.3. Critères de non inclusion

Les adultes et enfants survivants ou contacts présentant l'une des caractéristiques ou conditions suivantes ne pourront pas être inclus dans l'étude :

- Absence de suivi possible sur 12 mois d'un point de vue logistique ou géographique (uniquement pour les survivants) ;
- Non-résidents en RDC ;
- Impossibilité de se conformer aux exigences et procédures de l'étude selon l'opinion de l'investigateur ;
- Incapacité de consentir.

5.4. Modalités de recrutement

5.4.1. Population des survivants

Aujourd'hui la prise en charge des survivants est supervisée par le Programme National de suivi des guéris (PNSG). De nombreux survivants d'Ebola se sont déjà regroupés en association à travers l'« Association Nationale des Vainqueurs d'Ebola ». La prise en charge des guéris se fait au niveau de 4 sites dans les hôpitaux de Beni, Butembo, Mambasa et Mangina. Les survivants seront recrutés au niveau de ces 4 hôpitaux.

5.4.2. Population des contacts

Les personnes contacts seront identifiées par l'intermédiaire des survivants au moment de leur inclusion dans la cohorte des Vainqueurs d'Ebola avec la participation de l'Association Nationale des Vainqueurs d'Ebola. Il sera demandé aux patients guéris de la MVE d'informer les personnes contacts de leur unité domestique de l'étude sur les contacts, afin de faciliter le recrutement. A partir des listes de contacts établies lors du recrutement des survivants, les personnes contacts seront contactées et invitées pour un entretien avec un médecin investigateur. Les participants potentiels sont orientés ou référés au niveau des 4 centres de suivi du PNSG (hôpitaux de Beni, Butembo, Mambasa et Mangina) où le recrutement aura lieu. Un effort sera fait pour minimiser le temps entre l'exposition au cas malade et l'interrogatoire des contacts inclus, compte tenu néanmoins de la période d'observation de 21 jours des contacts.

6. MODALITES D'ORGANISATION DE LA RECHERCHE

Ce projet se calera sur l'organisation du PNSG et de l'INRB en renforçant la fréquence et la qualité du suivi en fonction des différents objectifs.

La faisabilité d'un tel projet est établie, les guéris bénéficiant déjà d'un cadre de suivi. Le fait que cette étude soit perçue par la plupart des acteurs comme un renforcement essentiel du PNSG jouera un rôle important également. Enfin, la qualité des procédures de suivi et de la prise en charge des personnes sera également déterminante. Il s'agira de constituer différents comités chargés de veiller à la bonne marche de l'étude en conformité avec les Bonnes Pratiques Cliniques et dans le respect de la législation nationale en vigueur.

TransVIHMI a acquis une solide expérience du suivi multidisciplinaire de cohorte de patients, et tout particulièrement dans le suivi des guéris de la MVE en Guinée (9,11).

6.1. Description du paquet d'accompagnement post CTE

Il s'agit d'un projet de recherche-action sur une période de 18 mois qui s'inscrit dans le cadre de la réponse française à l'épidémie d'Ebola. Il permettra la mise en place d'un véritable continuum des soins après la sortie d'un centre de traitement et cela de façon multidisciplinaire avec :

- La mise en place des moyens permettant un suivi au long cours des patients à travers une consultation médicale gratuite, la formation et le soutien aux personnels médicaux, médiateurs et associations,
- Le renforcement de capacité des sites des 4 hôpitaux participants afin de permettre la pérennisation d'une activité à long terme,
- La structuration d'un centre de recherche clinique au sein de l'INRB avec entre autres la mise en place d'un système de collecte et gestion de données ainsi qu'une équipe de monitoring.

L'ensemble de la prise en charge médicale (consultation, examens biologiques et complémentaires, médicaments, hospitalisations éventuelles) sera fourni gratuitement pendant toute la durée du suivi de l'étude. Le budget concernant le renforcement de structures et le transfert des compétences se fera de façon complémentaire au budget de la présente étude.

6.2. Sélection

6.2.1. Population des survivants

Les patients guéris de la MVE seront informés de l'étude au moment de leur sortie des CTE ou à travers l'association nationale des vainqueurs d'Ebola en RDC. Les participants potentiels sont orientés ou référés au niveau des 4 centres de suivi du PNSG.

6.2.2. Population des contacts

En moyenne, il est envisagé d'inclure 2 personnes-contacts par survivant inclus dans la cohorte des Vainqueurs d'Ebola. Ce qui fera une population cible estimée à 1 500 personnes.

6.3. Visite d'inclusion

Au niveau des trois centres du PNSG des hôpitaux de Beni, Butembo, Mambasa et Mangina et préalablement à la réalisation de tout examen clinique ou paraclinique spécifique à cette étude chez un patient guéri de la MVE ou un contact, le médecin investigateur vérifiera les critères d'éligibilité. Suite à cela, le consentement libre et éclairé de la personne doit être recueilli après qu'elle ait été informée par le médecin investigateur de l'objectif, du déroulement et de la durée de l'étude, des bénéfices, risques potentiels, contraintes et qu'elle aura pu poser toutes les questions éventuelles concernant l'étude. Il sera notamment précisé au participant ou à son parent/tuteur légal qu'il est entièrement libre de refuser ou d'arrêter sa participation ou celle de son enfant à tout moment sans encourir aucune sanction ni aucun préjudice de ce fait. Le retrait du contact d'un survivant à la MVE ne remettra pas en cause non plus le suivi du patient guéri inclus dans la cohorte des Vainqueurs d'Ebola. Le médecin investigateur s'assurera que les informations fournies sont claires, en utilisant le langage jugé le plus approprié, et facile à comprendre.

A l'issue de la séance d'information, le médecin investigateur remettra alors la notice d'information et le formulaire de consentement au participant ou à son parent/tuteur légal. Un délai de réflexion d'une semaine sera donné à chaque participant avant de donner sa réponse quant à sa participation à l'étude.

Si le participant et/ou son tuteur légal donne son accord, le formulaire de consentement sera produit en deux exemplaires datés et signés personnellement par celui-ci et l'investigateur ou le médecin qui le représente (une copie archivée par l'investigateur, une copie sera remise au patient ou à son parent ou représentant légal). Pour le mineur apte à exprimer sa volonté une adhésion à l'étude, de même que le consentement et l'assentiment devront être donnés à la fois par les titulaires de l'exercice de l'autorité parentale ainsi que par le mineur respectivement. Si un mineur atteint la majorité en cours d'étude, le processus de consentement sera initié à nouveau avec signature des deux exemplaires du consentement. Le consentement précédent sera conservé. Le consentement sera normalement recueilli par écrit (signature du formulaire). Si le consentement ne peut être donné par écrit, le consentement par empreintes digitales sera documenté officiellement. Après signature du consentement, un numéro d'identification unique est attribué à chaque participant.

Cette procédure d'information du participant et du recueil de consentement écrit, libre et éclairé est suivie pour toute nouvelle version de consentement émise en cours d'étude. En cas de nouvelle étude non prévue par le protocole initial, et après émission d'un amendement au protocole, soumis et accepté par le Comité d'Ethique pour la Recherche de l'Ecole de Santé Publique de Kinshasa, une nouvelle version de consentement est signée avant réalisation de cette nouvelle étude.

6.3.1. Population des survivants

La visite J0 correspond à la date de sortie du CTE et à la visite d'inclusion de l'étude. Toutefois, certains participants seront inclus après leur sortie du CTE. Dans ce cas, leur J0 correspondra à la date d'inclusion dans l'étude et les données à la sortie du CTE seront obtenues rétrospectivement et pourront ne pas comprendre la totalité des informations présentées ci-dessous. Lors de cette visite, les survivants doivent venir à jeun.

Au cours de la visite d'inclusion, seront effectués :

- le recueil des données sociodémographiques (âge, sexe, etc.), des antécédents biocliniques (y compris phase aiguë si disponible), médicaux et familiaux,
- un examen clinique complet de tous les systèmes et appareils comprenant les mesures anthropométriques (poids et taille),
- le recueil des signes et symptômes depuis la sortie du CTE par l'interrogatoire,
- un questionnaire standardisé et adapté à l'âge du patient permettant l'évaluer l'existence d'un syndrome dépressif :
 - o Le questionnaire CES-D pour les participants > 20ans
 - o Le questionnaire ADRS pour les participants entre 13 à 20 ans
 - o Le questionnaire CES-DC pour les participants entre 7 à 12 ans
 - o Le questionnaire PSYCa pour les participants entre 3 à 6 ans
- une prise de sang (adultes : 21 ml, enfants >7 kg : 10 ml, enfants < 7 kg : 5 ml) pour investigation paraclinique : lymphocytes totaux/CD4/CD8, NFS, CRP, ALAT, ASAT, créatinine, bilirubine, PCR EBOV (uniquement chez les survivants adultes participant à l'étude d'immunologie cellulaire), sérologie Ebola (IgG et IgM) et sérologie (VIH, VHB et VHC), analyses génétiques (ces dernières seront faites

chez les 50 premiers adultes ayant accepté de participer à l'étude génétique) ; une partie des prélèvements biologiques sera conservée pour des analyses ultérieures en relation avec l'étude,

- des prélèvements d'urine, salive, larmes, lait (femmes allaitantes), selles, si possible sécrétions vaginales (femmes âgées de plus de 15 ans) et sperme (hommes âgés de plus de 15 ans) en vue d'une recherche d'EBOV par PCR,

Une PCR sanguine pour EBOV sera effectuée chez les survivants ayant accepté de participer à l'étude d'immunologie cellulaire lors de l'inclusion. Si elle est négative, ils seront convoqués dans les 2 semaines suivants l'inclusion pour un prélèvement complémentaire de 44 ml de sang en vue d'analyses immunologiques complémentaires. Lorsque 60 vainqueurs adultes ayant reçu un traitement antiviral et 60 n'en ayant pas reçu lors de la phase aiguë de l'infection seront recrutés, les nouveaux inclus ne se verront plus invités à participer à cette étude.

6.3.2. Population des contacts

Au cours de la visite d'inclusion, seront effectués :

- Un questionnaire sociologique portant sur l'unité domestique,
- Le recueil des données démographiques des contacts (âge, sexe, etc.), et d'une éventuelle vaccination par un vaccin anti-Ebola (preuve et date),
- L'identification précise du/des cas guéris inclus dans la cohorte des Vainqueurs d'Ebola auquel a été exposé le cas contact,
- Le recueil des signes et symptômes des contacts par interrogatoire depuis l'exposition aux malades ayant présenté une MVE,
- Une prise de sang (adultes 7 ml, enfants 3 ml) pour la recherche d'anticorps anti-EBOV, donnée également utilisée pour les sous études immunologie et génétique, pour la recherche d'EBOV par PCR (contacts adultes participant à l'étude d'immunologie cellulaire), l'étude génétique (cette dernière sera faite chez les 100 premiers contacts adultes ayant accepté de participer à l'étude génétique – 50 ne présentant pas d'anticorps anti-Ebola et 50 présentant des anticorps anti-Ebola). Une partie du sérum sera conservée pour des analyses ultérieures en relation avec l'étude.
- Une PCR sanguine pour EBOV sera effectuée chez les contacts ayant accepté de participer à l'étude d'immunologie cellulaire lors de l'inclusion et qui ont une sérologie EBOV négative. Si la PCR EBOV est également négative, ils seront convoqués avec les autres participants de l'étude d'immunologie cellulaire ayant une sérologie négative à l'inclusion dans les 2 semaines suivants l'inclusion pour un prélèvement complémentaire de 44 ml de sang pour les analyses de l'étude d'immunologie cellulaire. Lorsque 40 contacts adultes vaccinés contre Ebola et 40 non vaccinés seront recrutés, les nouveaux inclus ne se verront plus invités à participer à cette étude.

En cas de sérologie positive pour le virus Ebola, des prélèvements d'urine, salive, lait (femmes allaitantes), larmes, selles, si possible sécrétions cervico-vaginales (femmes âgées de plus de 15 ans) et sperme (hommes âgés de 15 ans et plus) en vue d'une recherche du virus Ebola par PCR seront effectués lors d'une seconde visite dans les 2 semaines suivants la visite d'inclusion et seront référés au PNSG.

6.4. Visite de suivi

Les visites de suivi concernent les survivants. Si les contacts présentent des anticorps anti-EBOV, ils seront testés pour la recherche de virus dans leur liquide biologique puis seront référés au PNSG.

6.4.1. Population des survivants

Un suivi bioclinique de routine des survivants sera réalisé à un mois, à trois mois, à six mois, à neuf mois et à douze mois au niveau des quatre centres du PNSG des hôpitaux de Beni, Butembo, Mambasa et Mangina. Si le dernier participant n'a pas atteint sa visite à 12 mois et qu'un participant atteint les 18 mois après inclusion, une visite lui sera proposée à cette date (M18). Durant les visites de suivi, les investigations suivantes seront réalisées :

- un examen clinique complet de tous les systèmes et appareils comprenant les mesures anthropométriques (poids et taille),
- le récapitulatif des pathologies survenues depuis la dernière échéance du protocole par l'interrogatoire,
- un questionnaire standardisé et adapté à l'âge du patient permettant l'évaluer l'existence d'un syndrome dépressif (pour les visites J0, M3, M12, uniquement):
 - o Le questionnaire CES-D pour les participants > 20ans
 - o Le questionnaire ADRS pour les participants entre 13 à 20 ans
 - o Le questionnaire CES-DC pour les participants entre 7 à 12 ans
 - o Le questionnaire PSYCa pour les participants entre 3 à 6 ans
- une prise de sang (adultes : 21 ml, enfants >7 kg : 10 ml, enfants < 7 kg : 5 ml) pour examen paraclinique : lymphocytes totaux/CD4/CD8, NFS, CRP (ces 3 premiers tests ne sont fait qu'à M1, M6, M12 et M18 uniquement), ALAT, ASAT, créatinine, bilirubine, PCR EBOV (uniquement chez les survivants adultes participant à l'étude d'immunologie cellulaire à M6 et M12), sérologie Ebola (IgG et IgM), analyses génétiques (chez les 50 premiers adultes ayant accepté de participer à l'étude génétique) ; une partie des prélèvements biologiques sera conservée pour des analyses ultérieures en relation avec l'étude,
- des prélèvements d'urine, salive, larme, lait (femmes allaitantes), selles, si possible sécrétions vaginales (femmes âgées de plus de 15 ans) et sperme (hommes âgés de 15 ans et plus) en vue d'une recherche d'EBOV par PCR.

Les 120 survivants adultes inclus dans l'étude d'immunologie cellulaire auront un prélèvement complémentaire de 44 ml de sang en vue de l'analyse immunologique cellulaire dans les 2 semaines après les visites M6 et M12 si la PCR EBOV est négative lors de la visite M6 et M12 précédente.

A l'issue de chaque visite, le médecin investigateur planifie le rendez-vous pour la visite suivante en rappelant au participant, son parent/tuteur légal de la nécessité d'être à jeun pour la prise de sang aux visites M1, M6, M12 et M18. Les conditions de prélèvements des sécrétions vaginales et du sperme seront expliquées en détail dans le manuel de procédure laboratoire.

Un contact téléphonique sera pris par l'équipe du centre d'étude au cours des jours précédant la visite pour rappeler la date de rendez-vous.

	Inclusion	Suivi					
	J0	M1	M3	M6	M9	M12	M18 ¹
Signature du consentement éclairé	X						
Recueil des données sociodémographiques, cliniques et des antécédents	X						
Examen physique complet avec anthropométrie poids et taille	X	X	X	X	X	X	X
Interrogatoire sur les signes et symptômes	X	X	X	X	X	X	X
Questionnaire sur le syndrome dépressif	X		X			X	
Bilan biologique sanguin (21 ml de sang²)	X	X	X	X	X	X	X
Lymphocytes totaux/CD4/CD8, NFS et CRP	X	X		X		X	X
ALAT, ASAT, créatinine, bilirubine	X	X	X	X	X	X	X
PCR EBOV ³	X			X		X	
Sérologie anti EBOV (IgG et IgM)	X	X	X	X	X	X	X
Sérologie (VIH, VHB, VHC)	X						
Stockage au long-terme des aliquots de plasma (7 ml) avec buffy coat ⁴	X	X	X	X	X	X	X
Bilan biologique autre	X	X	X	X	X	X	X
PCR EBOV (urine, salive, larmes, lait (femmes allaitantes), selles, si possible sécrétions vaginales (femmes âgées de plus de 15 ans) et sperme (hommes âgés de 15 ans et plus)	X	X	X	X	X	X	X
Etude immunologique cellulaire (44 ml de sang) dans les 2 semaines après la visite de suivi³	X			X		X	

¹La visite M18 sera proposée aux participants jusqu'à ce que le dernier participant inclus atteigne la visite M12. Après ce moment, la visite M18 ne sera plus effectuée.

²Chez l'enfant, le volume à adapter selon le poids et l'état clinique (5 ml pour les enfants de moins de 7 kg et un minimum de 10 ml pour les plus de 7 kg).

³Uniquement chez les 120 adultes pour l'étude immunologique. Les survivants seront convoqués pour une visite dans les 2 semaines suivant la visite de suivi protocolaire. Ils ne seront prélevés que si la PCR sanguine pour EBOV est négative.

⁴Des analyses génétiques seront effectuées chez 50 vainqueurs adultes.

6.5. Sortie d'étude et perdus de vue

6.5.1. Population des survivants

Chaque participant peut sortir quand il le souhaite de cette étude et continuer à bénéficier du soutien du PNSG. Au niveau de chaque centre participant, l'association nationale des Vainqueurs d'Ebola assurera le soutien aux participants et la recherche des perdus dans le but d'aider à obtenir des informations sur la cause d'une sortie d'étude : retrait du consentement, refus du suivi, déménagement, décès, etc.

Afin de minimiser le taux de perte le cas échéant, une démarche de suivi multiple sera mise en place :

- Collecte d'informations détaillées permettant de localiser (adresse, numéro de téléphone, etc.) chaque participant lors de la visite d'inclusion,
- Mise à jour de l'adresse et du numéro de téléphone lors de chaque visite de suivi,
- Contact téléphonique avec les participants ou leurs parent(s)/tuteur légal au cours des jours précédant la date de la prochaine visite protocolaire et en cas de visite manquée,
- Contact téléphonique avec une tierce personne (personne de confiance choisie par le participant) si impossibilité de joindre ou retrouver un participant dans l'étude,
- Maintien d'une communication régulière avec les participants ou leur(s) parents/tuteur légal afin d'accroître la sensibilisation sur les thématiques et les objectifs de l'étude et de l'importance de participer à toutes les visites de suivi.

A la fin de l'étude, si un participant n'est plus suivi depuis plus de six mois malgré les tentatives de l'équipe d'origine pour reprendre contact avec lui et sans qu'aucune cause ne soit repérée, il sera alors considéré comme perdu de vue.

6.5.2. Population des contacts

Aucune sortie d'étude n'est prévue pour les contacts car il s'agit d'une enquête transversale.

7. COLLECTION D'ÉCHANTILLONS BIOLOGIQUES D'ORIGINE HUMAINE

Dans le cadre de la lutte contre l'épidémie, toutes les activités de laboratoire sont supervisées par l'INRB et les prélèvements sont stockés à l'INRB à Kinshasa. L'étude suivra l'organisation actuelle.

7.1. Conditions de prélèvement

Au niveau des quatre centres du PNSG des hôpitaux de Beni, Butembo, Mamabassa et Mangina, des échantillons de sang, d'urine, larmes, de salive, de lait, de selles, des sécrétions vaginales et de sperme seront collectés aux différentes visites protocolaires (cf. section 6.4) pour permettre la réalisation des examens biologiques en temps réel et le stockage d'une partie des échantillons prélevés en vue des analyses ultérieures en relation avec l'étude. Pour les bilans biologiques, hématologiques et biochimiques, les techniques et "trousses" utilisées seront celles des hôpitaux en charge du suivi des guéris.

7.1.1. Population des survivants

Deux tubes EDTA et 1 tube sec seront remplis par 7 ml de sang pour un total de 21 ml par visite chez les adultes. Chez les enfants de plus de 7 kg, le volume total de sang prélevé sera de 10 ml (7 ml en tube EDTA et 3 ml en tube sec). Chez les enfants de moins de 7 kg, ce volume sera de 5 ml (3,5 ml en EDTA et 1,5 ml sur tube sec). Les autres prélèvements biologiques seront effectués à toutes les visites dans un pot à coproculture pour les selles, dans un pot à urine pour la salive (prélèvement minimal de 1 ml) et les urines, dans un flacon stérile pour

le sperme (hommes âgés de 15 ans et plus) et le lait maternel (femmes allaitantes), par un écouvillon placé dans un milieu de transport (RNA later) fermé pour les sécrétions cervico-vaginales (femmes âgées de 15 ans et plus) et par écouvillon pour les larmes.

Les 120 participants adultes participant à l'étude immunologique auront un prélèvement complémentaire de 44ml de sang dans 4 tubes EDTA de 9 ml, 1 tube sec de 5 ml et 1 tube tempus de 3 ml dans les 2 semaines suivantes les visites J0, M6 et M12, si la PCR sanguine est négative pour EBOV à la visite J0, M6 et M12 précédente. Les tubes nécessaires à l'étude immunologique seront préparés (isolation des PBMC et du sérum) et stockés à -80°C dans les laboratoires de l'INRB avant rapatriement au sein de la plateforme d'immunomonitoring du Vaccine Research Institute (VRI), Créteil, France en vue des analyses d'immunologie cellulaire.

7.1.2. Populations des contacts

Un tube EDTA sera utilisé pour prélever un total de 7 ml de sang à la visite d'inclusion chez les adultes ou 3 mL chez les enfants.

Chez les contacts ayant une sérologie à EBOV positive, des prélèvements biologiques seront effectués dans les deux semaines suivant la visite d'inclusion dans un pot à coproculture pour les selles, dans un pot à urine pour la salive (prélèvement minimal de 1 ml) et les urines, dans un flacon stérile pour le sperme et le lait maternel, par un écouvillon placé dans un milieu de transport (RNA later) fermé pour les sécrétions cervico-vaginales et par écouvillon pour les larmes.

Les 80 participants adultes participant à l'étude immunologique auront un prélèvement complémentaire de 44 ml de sang dans 4 tubes EDTA de 9 ml, 1 tube sec de 5 ml et 1 tube tempus de 3 ml dans les 2 semaines suivantes la visite d'inclusion si la PCR sanguine est négative pour EBOV lors de la visite d'inclusion.

Les tubes nécessaires à l'étude immunologique seront préparés (isolation des PBMC et du sérum) et stockés à -80°C dans les laboratoires de l'INRB avant rapatriement au sein de la plateforme d'immunomonitoring du VRI, Créteil, France en vue des analyses immunologiques.

7.2. Modalités de codage et d'étiquetage

Tous les prélèvements et échantillons réalisés sont codés et ne sont donc pas nominatifs.

Les aliquots conservés au congélateur à -20°C ou à -80°C pour ceux de l'étude d'immunologie cellulaire seront marqués avec des étiquettes spéciales congélation qui seront complétées à l'aide d'un stylo dont l'encre est résistante à la congélation. Ces étiquettes auront été préalablement testées pour vérifier leur maintien après des cycles de congélation/décongélation successifs avant toute utilisation dans le cadre de l'étude. Les étiquettes comporteront le code de l'étude, le numéro de visite, le N° d'identification du patient dans l'étude, la date du/des prélèvement(s) et la nature du prélèvement (plasma). Le devenir des échantillons sera expliqué dans la lettre d'information au participant ou au parent/tuteur légal. Ces analyses complémentaires ne feront donc pas l'objet d'un consentement distinct.

7.3. Traitement des échantillons

7.3.1. Population des survivants

Echantillons de la cohorte prélevés à toutes les visites :

Echantillon	Volume utilisé par analyse	Conservation
Adulte : sang, 2x 7 ml tube EDTA	2 ml lymphocytes ¹ au niveau du site de suivi	Destruction après analyse
Enfant > 7 kg : Sang, 7 ml tube EDTA	1 ml NFS ¹ au niveau du site de suivi	Destruction après analyse
	0,2 ml CRP ¹ au niveau du site de suivi	Destruction après analyse
Enfant < 7 kg : Sang, 3,5 ml tube EDTA	0,2 ml ALAT au niveau du site de suivi	Destruction après analyse
	0,2 ml ASAT au niveau du site de suivi	Destruction après analyse
	0,2 ml créatinine au niveau du site de suivi	Destruction après analyse
	0,2 ml bilirubine au niveau du site de suivi	Destruction après analyse
	2 ml PCR au niveau du site de suivi à J0, M6 et M12 pour les survivants dans l'étude d'immunologie cellulaire	Destruction après analyse
	1 ml sérologie VIH, VHB, VHC ² au niveau du site de suivi	Destruction après analyse
	1 ml sérologie anti EBOV au niveau du site de suivi	Destruction après analyse
Adulte : sang, 7 ml (adulte) tube sec	Echantillon de sérum pour stockage au long-terme.	Conservation -20°C au niveau des sites puis stockage à -20°C à l'INRB de Kinshasa.
Enfant > 7 kg : Sang, 3 ml tube sec	Pour les participants à l'étude génétique, le buffy coat sera récupéré (0,5 ml)	A partir du buffy coat l'ADN sera extrait à l'INRB de Kinshasa, puis stocké à -20°C avant d'être envoyé en France où il sera détruit après analyse
Enfant < 7 kg : Sang, 1,5 ml tube sec		
Selles (pot), salive (≥ 1 ml pot), larmes (écouvillon), urine (pot), sperme (flacon stérile), lait maternel (flacon stérile), sécrétions cervico-vaginales (écouvillon)	Totalité du prélèvement pour PCR EBOV au niveau du site de suivi	Conservation -20°C de la partie du prélèvement restant après analyse à l'INRB de Kinshasa

¹Uniquement pour les visites J0, M1, M6, M12 et M18

²Uniquement pour la visite d'inclusion

Echantillon complémentaire dans les 2 semaines suivants les visites J0, M6 et M12 uniquement pour les participants inclus dans l'étude immunologique cellulaire (n=120) :

Echantillon	Volume utilisé par analyse	Conservation
Sang, 4x 9 ml tube EDTA	Préparation de 4 aliquots de PBMC pour analyse Intracellular Staining (ICS) et de phénotype en France	PBMC stockées à -80°C à l'INRB de Kinshasa avant l'envoi en France, Destruction après analyse
Sang, 5 ml tube sec	4 ml utilisés pour la préparation de 4 aliquots de 200 µl pour analyse Luminex en France	Sang stocké à -80°C à l'INRB de Kinshasa avant l'envoi en France, destruction après analyse
Sang, 3 ml tube tempus	3 ml pour analyse du profil génique en France	Sang stocké à -80°C à l'INRB de Kinshasa avant l'envoi en France, destruction après analyse

7.3.1. Population des contacts

Echantillons enquête transversale à la visite d'initiation :

Echantillon	Volume utilisé par analyse	Conservation
Sang, 7 ml (adulte) ou 3 ml (enfant) tube EDTA	2 ml sérologie anti EBOV (Luminex)	Destruction après analyse
	1 ml (buffy coat) analyse génétique chez 100 participants adultes envoyé en France	Conservation -20°C au niveau des sites puis stockage à -20°C à l'INRB de Kinshasa. A partir du buffy coat l'ADN sera extrait à l'INRB de Kinshasa, puis stocké à -20°C avant d'être envoyé en France où il sera détruit après analyse
	Sérum restant pour le stockage au long-terme	Conservation -20°C à l'INRB de Kinshasa
Selles (pot), salive (≥ 1 ml pot), larmes (écouvillon), urine (pot), sperme (flacon stérile), lait maternel (flacon stérile), sécrétions cervico-vaginales (écouvillon) ¹	Totalité du prélèvement pour PCR EBOV	Conservation -20°C à l'INRB de Kinshasa

¹Uniquement chez les contacts ayant une sérologie anti-EBOV positive, prélèvements effectués lors d'une seconde visite dans les 2 semaines suivant l'inclusion

Echantillon complémentaire uniquement pour les participants de l'étude immunologique cellulaire dans les 2 semaines suivants la visite d'inclusion (n=80) :

Echantillon	Volume utilisé par analyse	Conservation
Sang, 4x 9 ml tube EDTA Utilisé pour la préparation de aliquots de PBMC	Préparation de 4 aliquots de PBMC pour analyse Intracellular Staining (ICS) et de phénotype en France	PBMC stockées à -80°C à l'INRB de Kinshasa avant l'envoi en France, destruction après analyse
Sang, 5 ml tube sec	4 ml utilisés pour la préparation de 4 aliquots de 200 µl pour analyse Luminex en France	Sang stocké à -80°C à l'INRB de Kinshasa avant l'envoi en France, destruction après analyse
Sang, 3 ml tube tempus	3 ml pour analyse du profil génique en France	Sang stocké à -80°C à l'INRB de Kinshasa avant l'envoi en France, destruction après analyse

7.4. Transport

Après prélèvement, les échantillons seront acheminés au niveau des laboratoires des hôpitaux de Beni, Mangina, Mambasa et Butembo où seront effectuées les analyses et la conservation avant envoi à l'INRB de Kinshasa puis en France exclusivement pour les études complémentaires immunologiques et génétiques.

Les échantillons de l'étude immunologique seront envoyés en carboglace à -80°C à Créteil, France, au Vaccine Research Institute tous les 3 à 6 mois.

Les échantillons de l'étude génétique (ADN) sur 50 vainqueurs adultes et 100 contacts adultes seront transportés en une fois à -20°C au laboratoire de génétique humaine des maladies infectieuses, Paris, France. Avant tout envoi en France, une autorisation d'importation délivrée par le ministère de la recherche et de l'enseignement supérieur français devra être obtenue pour les échantillons de l'étude immunologique et génétique.

7.5. Stockage et/ou destruction

Les échantillons de l'étude d'immunologie cellulaire seront stockés à -80°C au niveau des hôpitaux de suivi puis à l'INRB de Kinshasa avant d'être envoyé au Vaccine Research Institute où ils seront analysés et détruits après analyse.

Les autres échantillons biologiques seront stockés à -20°C au niveau des hôpitaux de suivi puis à l'INRB à Kinshasa comme cela se fait actuellement dans le cadre du PNSG. Ils seront conservés 10 ans après la fin de l'étude, mis à part les prélèvements sur tube EDTA qui seront détruits après analyse. Chez 50 adultes parmi les survivants et 100 adultes parmi les contacts, l'ADN extrait du buffy coat sera envoyé au laboratoire de génétique humaine des maladies infectieuses en France pour l'analyse génétique ; ces échantillons seront détruits après analyse.

7.6. Description du système qualité de la collection

Au niveau des hôpitaux, le suivi des échantillons biologiques se fera au format papier. Une application « base de données biothèque » sera créée pour la gestion informatique de tous les échantillons biologiques conservés

à la bibliothèque de l'INRB de Kinshasa et sous sa responsabilité et selon la législation en vigueur en RDC. Une base de données informatique sera également utilisée au niveau des laboratoires en France.

8. RECUEIL ET TRAITEMENT DES DONNEES

8.1. Description des données recueillies

Nature des données	Données recueillies dans le cadre du projet (oui/non)
Identification par patronyme complet	non
Identification par numéro de centre et numéro d'ordre	oui
Autre mode d'identification (code aléatoire à quatre lettres)	oui
Données démographiques (âge et sexe)	oui
Santé (ex : antécédents personnels, familiaux, traitements...)	oui
Données symptomatiques et données relatives aux soins	oui
Situation familiale (ex : seul, en couple...)	oui
Situation militaire	non
Niveau de formation-diplômes (ex : niveau scolaire, formation professionnelle)	oui
Situation professionnelle (ex : actif, chômeur, catégories socioprofessionnelles...)	oui
Situation économique et financière (ex : niveau de revenus, prestations sociales...)	non
Consommation des biens et services (ex : aide à domicile, transport...)	non
Habitudes de vie, comportement (ex : consommation d'alcool, de tabac, pratiques sportives...)	non
Numéro de sécurité sociale ou RNIPP	non
Origines raciales ou opinions politiques, philosophiques, religieuses, appartenances syndicales ou mœurs	non
Prélèvements biologiques non identifiants (avec code anonyme)	non
Infractions, condamnations ou mesures de sûreté	non

8.1.1. Population des survivants

Les données recueillies au cours des différentes visites seront :

- Données sociodémographiques : âge, sexe, situation familiale,
- Santé : antécédents biocliniques, médicaux, familiaux et psychologiques, traitements médicaux concomitants, résultats des analyses biologiques et des examens cliniques, poids, taille, résultats d'analyse génétique.

8.1.2. Population des contacts

Les données recueillies au cours des différentes visites seront :

- Données sociodémographiques : âge, sexe, situation familiale,

- Santé : antécédents de vaccination contre la MVE, antécédents médicaux (symptômes de MVE) résultats des analyses biologiques, résultats d'analyse génétique,
- Questionnaire sociologique sur l'unité domestique, sur le cas index et sur l'exposition du contact.

8.2. Définition des données sources

Les participants n'étant pas hospitalisés, il n'existe pas de dossier médical. Le recueil des données sera donc effectué directement à l'aide de cahiers d'observation papiers standardisés attribués à chaque participant inclus dans l'étude. Les autres données sources sont les résultats des analyses biologiques au format papier.

8.3. Circuit des données

Les informations seront directement reportées dans le cahier d'observation au format papier lors des visites de suivi. Les résultats d'analyse biologique seront imprimés puis reportés sur le cahier d'observation papier. Les différents questionnaires seront intégrés directement au cahier d'observation. Ces différents documents ne comporteront pas le nom de la personne mais uniquement son identifiant et seront conservés dans un classeur rangé dans une pièce fermée à clef accessible uniquement à l'attaché de recherche clinique lorsqu'il n'est pas utilisé pour les visites d'inclusion et de suivi ou le renseignement de la base de donnée informatisée. Une fiche de correspondance sera établie entre l'identifiant et le participant qui sera gardée à l'INRB et au niveau des différents sites, uniquement accessible aux personnes autorisées et désignées par le CMG.

Un ensemble de consignes, facilitant le remplissage des fiches du classeur, la gestion et la logistique de l'étude sera intégré au classeur d'observation. Chaque investigateur devra compléter et signer les feuillets du cahier d'observation qui le concernent. Ces cahiers devront être complétés de façon lisible au stylo à bille, chaque correction effectuée devant être datée et signée par le médecin investigateur, la donnée initiale devant rester visible.

La vérification de la qualité des données sera effectuée par l'assistant de recherche clinique affecté à cette étude qui recherchera l'existence de données manquantes et aberrantes. Toutes données erronées ainsi retrouvées, feront l'objet d'une demande de correction par l'investigateur du site d'étude ayant adressé le cahier d'observation. Une double saisie des données sur ordinateur sera effectuée ensuite par deux agents de saisie. Elles seront transmises à une base de données RedCAP hébergée à l'INRB de Kinshasa. Cette base sera accessible uniquement par les personnes autorisées par le CMG et verrouillée par un mot de passe individuel de 6 caractères au minimum. En cas de correction des données, la traçabilité (personne ayant modifiée et valeur originale) sera assurée. Cette base de données sera sauvegardée quotidiennement sur un disque dur externe gardé sous clef et hebdomadairement sur un support différent conservé sous clef en dehors du centre d'inclusion. Les clefs USB flash seront interdites. La base de données sera synchronisée chaque nuit sous la forme d'un dump vers un serveur hébergé au CMG de TransVIHMI à l'IRD de Montpellier. Cet envoi se fera via le protocole sécurisé SSH FTP (Secure File Transfer Protocol) en utilisant 2 clés de cryptage. Le CMG effectuera une vérification automatique des données à chaque exportation et renverra aux investigateurs du projet une liste de « *queries* » pour action. La collecte et la gestion des données de l'étude des contacts utilisera l'application déjà développée pour l'étude des contacts de Guinée (Contactebogui).

8.4. Conservation des documents de la recherche

Les documents relatifs à la recherche sont archivés conformément à la réglementation en vigueur.

Le promoteur et les investigateurs conservent les documents relatifs à la recherche, qui leur sont spécifiques pendant une durée de 10 ans au niveau de l'INRB de Kinshasa et du CMG de TransVIHMI.

Aucun déplacement ou destruction ne peut se faire sans l'accord du promoteur. Au terme de la durée réglementaire d'archivage, le promoteur sera consulté pour destruction.

Toutes les données, tous les documents et les rapports peuvent faire l'objet d'audit ou d'inspection.

9. VIGILANCE DE LA RECHERCHE

9.1. Définitions

Un effet indésirable est défini comme toute manifestation nocive et non souhaitée susceptible d'être liée à la manipulation ou à la consommation d'un produit, d'une substance ou survenue lors d'un acte de soins.

Un effet indésirable répond à la définition de "grave" s'il:

- entraîne la mort;
- met en danger la vie du participant (effet au cours duquel le sujet risquait de décéder; il ne désigne pas un événement/effet qui aurait hypothétiquement pu causer la mort s'il avait été plus grave);
- nécessite une hospitalisation ou la prolongation de l'hospitalisation;
- provoque un handicap ou une incapacité importants ou durables;
- se traduit par une anomalie ou malformation congénitale;
- est un "événement médical important" (événement considéré par l'investigateur comme médicalement important et pouvant mettre en danger le sujet ou nécessiter une intervention, médicale ou chirurgicale, pour prévenir l'une des caractéristiques/conséquences mentionnées ci-dessus. Exemples: traitement intensif aux urgences hospitalières ou au domicile du participant à la recherche pour un bronchospasme allergique, une crise convulsive ou des troubles de la coagulation).

9.2. Responsabilités de l'investigateur

L'investigateur déclare au promoteur tout effet indésirable grave **susceptible d'être lié aux procédures de la recherche (ici lié aux prises de sang et aux diverses prélèvements) et tout fait nouveau**, c'est-à-dire toute nouvelle donnée de sécurité pouvant conduire à une réévaluation du rapport bénéfice/risque de la recherche, ou qui pourrait être suffisant pour envisager des modifications des documents relatifs à la recherche.

Pour cela, il envoie sans délai une copie de la section du cahier d'observation détaillant les circonstances de l'effet indésirable grave ou du fait nouveau à l'adresse pharmacovigilance.prc@inserm.fr.

Tout autre effet indésirable susceptible d'être lié à un produit pris par le participant doit être déclaré par l'investigateur aux structures compétentes (avec copie au promoteur) selon la réglementation applicable en République Démocratique du Congo.

9.3. Risques potentiels de la recherche et prise en charge médicale en cas de survenue d'effet indésirable

Les risques potentiels liés à la prise de sang sont :

- Léger inconfort ou une douleur locale et/ou une inflammation et/ou une formation d'hématome au niveau de la peau où l'aiguille est insérée dans la veine.
- Des infections localisées peuvent survenir à l'endroit où la veine a été perforée (très rarement)

- Des maux de tête, des nausées et des vomissements peuvent survenir quelques heures après la ponction veineuse (très rarement).

Toutes les mesures d'hygiène hospitalières appropriées seront mises en place pour prévenir l'apparition de ces effets.

10. ANALYSE STATISTIQUE DES DONNEES

10.1. Responsable de l'analyse statistique

Jean-François Etard et Sandrine Leroy de TRANSVIHMI (Inserm-IRD-UMI) seront responsables de l'analyse statistique.

10.2. Taille de la population de l'étude

Au 11/04/2020, 1169 personnes ont été déclarées « guéries » et notre mode d'inclusion dynamique impliquant les personnes dès leur sortie d'un CTE au jour de la mise en place de l'étude et ceux déjà sorties (« late entry ») rend réaliste et faisable notre objectif de 800 personnes à inclure.

L'expérience de l'étude sur les contacts en Guinée (802 survivants/1721 contacts) montre que l'on peut raisonnablement inclure 2 personnes-contacts/survivant. Compte tenu de la dynamique de l'épidémie et de la forte incidence actuelle, il est prévu d'inclure 1500 contacts. Selon les données les plus récentes issues des études en Afrique de l'ouest, un taux de positivité des anticorps anti-EBOV de 5% est prévisible. Entre 3.9%- 6.2% (IC 95%) des 1500 contacts auront des anticorps anti-EBOV.

L'étude immunologique cellulaire comprendra une sous-population de 200 personnes incluses dans cette étude : 60 survivants ayant reçu un traitement antiviral pendant la phase aigüe de l'infection, 60 survivants n'ayant pas reçu de traitement antiviral ainsi que 40 contacts non infectés non vaccinés et 40 contacts non infectés et vaccinés.

L'étude génétique comprendra une sous-population de 150 personnes incluses dans cette étude : 50 personnes contacts non infectés (absence d'anticorps anti-nucléoprotéine, anti-glycoprotéine ou anti-VP40 d'EBOV) et présentant un haut risque d'exposition au virus (9) 50 personnes contacts infectées (c'est-à-dire asymptomatiques ou pauci-symptomatiques et présentant des anticorps contre au moins deux antigènes d'EBOV (nucléoprotéine, glycoprotéine ou VP40 d'EBOV) et 50 personnes survivantes présentant les formes les plus graves de la maladie et disposant des informations cliniques pertinentes disponibles (par exemple, charge virale).

10.3. Description du plan d'analyse statistique

10.3.1. Analyse de la cohorte des survivants

Une analyse descriptive de l'ensemble de la population recrutée sera effectuée afin de vérifier s'il existe des écarts au protocole soit au moment de l'inclusion soit en cours de suivi. Seront mentionnés le pourcentage de patients inclus à tort (leurs données ne seront pas analysées) et le pourcentage de sujets perdus de vue (les données obtenues seront analysées). Des analyses descriptives en sous-groupes seront faites : comparaison

en fonction des traitements reçus pendant l'hospitalisation, en fonction du tableau clinique (charge virale EBOV) ou en fonction de l'âge (comparaison entre adultes et enfants). Les variables quantitatives seront décrites par leur effectif, moyenne, écart-type, médiane, 1^{er} et 3^{ème} quartiles, valeurs extrêmes et nombre de données manquantes. Les variables qualitatives seront décrites par leur effectif, pourcentage et nombre de données manquantes par modalité de réponse.

Un premier bilan des inclusions est prévu après 1 mois, puis 3 et 6 mois. Une analyse intermédiaire des données est prévue à 12 mois.

La proportion de patients présentant des séquelles, une co-infection avec le VIH et l'hépatite B, des anticorps ou un ARN EBOV dans les différents fluides organiques, seront rapportées. Des tests paramétriques et éventuellement non paramétriques seront utilisés pour évaluer la cinétique de la clairance virale dans les fluides organiques, des lymphocytes T CD4/CD8 et des anticorps.

Une analyse de survie sera utilisée pour modéliser :

1. Le temps de clairance virale dans les fluides organiques, c'est-à-dire la période entre la guérison clinique et le moment à partir duquel EBOV n'est plus détecté dans aucun fluide organique.
2. Le temps de disparition des séquelles, c'est la période entre la fin de l'hospitalisation du patient et la disparition complète de toute séquelle attribuée à la MVE sans réapparition au cours des visites postérieures.

Les co-variables d'intérêt principales seront principalement mais non-exhaustivement l'âge, le sexe, le traitement reçu, la sévérité de l'infection initiale représentée par la charge virale initiale mesurée par PCR (reflétée le nombre de Ct) au moment du diagnostic et les co-morbidités initiales.

Les modèles de survie paramétriques seront ajustés pour estimer la probabilité de clairance virale ou de disparition des séquelles au cours du temps en utilisant une distribution gamma. La méthodologie utilisée est décrite en détail ailleurs (Subtil F, Delaunay C, Keita AK, et al. Dynamics of Ebola RNA Persistence in Semen: A Report From the Postebogui Cohort in Guinea. Clin Infect Dis [Internet]. 2017; 64(12):1788–1790. Available from: <https://academic.oup.com/cid/article-lookup/doi/10.1093/cid/cix210>).

Le seuil global des tests sera de 5% et les analyses seront réalisées sur les logiciels Stata 15 et R.

10.3.2. Analyse des contacts

L'unité statistique est le sujet contact.

Une analyse descriptive de l'ensemble des personnes contacts recrutées sera effectuée, afin de décrire l'exposition, les caractéristiques cliniques (formes asymptomatiques ou pauci symptomatiques), la prévalence des personnes contacts avec anticorps anti-EBOV, la distribution des réponses anticorps vis-à-vis des trois antigènes (NP, GP, VP40) du test sérologique (Luminex), la proportion de sujets contacts avec une présence de virus dans les fluides corporels. Les variables quantitatives seront décrites par leur effectif, moyenne, écart-type, médiane, 1^{er} et 3^{ème} quartiles, valeurs extrêmes et nombre de données manquantes. Les variables

qualitatives seront décrites par leur effectif, pourcentage et nombre de données manquantes par modalité de réponse.

Une analyse intermédiaire des données est prévue après l'inclusion des 500 premiers contacts, et permettra de donner une première estimation de la prévalence des sujets contacts séro-convertis, et de revoir si besoin la taille de l'enquête en fonction.

La description des caractéristiques des sujets contacts se fera ensuite par sous-groupes : en fonction de la sérologie anti-EBOV, et selon le niveau d'exposition recueilli. Des tests paramétriques et éventuellement non paramétriques seront utilisés pour comparer les variables continues entre les différents sous-groupes. Des tests de χ^2 (éventuellement de Fischer en cas de petits effectifs) ou des tests de Cochran seront utilisés pour comparer les variables binaires et ordinales respectivement entre les différents sous-groupes.

A partir des données de l'enquête transversale, des analyses multivariées seront réalisées pour l'analyse des différents critères de jugement permettant un ajustement sur des covariables d'intérêt (âge, sexe, niveau d'exposition) par des modèles de régression logistique pour les variables dépendantes binaires (séro- conversion, etc.) Le but sera d'identifier des facteurs associés à la séro-conversion. L'exposition sera prise en compte dans les modèles multivariés d'une part par les variables individuelles (nature/type de l'exposition) d'exposition et d'autre part en utilisant le « proxy » du niveau d'exposition défini ci-dessus, ce qui n'imposera pas le recours à des modèles de données corrélées pour l'analyse. Les analyses étudiant les relations entre séropositivité et niveau d'exposition seront aussi menées en utilisant des modèles à classes latentes. Ceci permettra de vérifier par une approche statistique différente les relations entre la séropositivité et l'exposition aux malades avec une MVE.

Il pourra être pertinent, et sera discuté, de tirer parti de la dépendance des données d'exposition entre les sujets contacts qui « partagent » les mêmes malades auxquels ils ont été exposés. Ceci pour vérifier, et éventuellement implémenter en cas de données manquantes les informations collectées chez différents sujetscontacts pour le même malade auquel ils ont été exposés.

Le seuil de 0,20 sera utilisé pour la sélection des variables en analyse univariée pour les prendre en considération dans les modèles multi-variés. Le seuil global des tests sera de 5% et les analyses seront réalisées sur les logiciels Stata® 15 et R®.

10.3.3. Réponse immunitaire humorale spécifique

Les données xMAP générées seront log-transformées, analysées globalement et stratifiées par points temporels et par genre. Nous comparerons la proportion de chaque isotype d'IgG aux différents moments de la cohorte de survivants pour évaluer l'évolution dans le temps. Nous comparerons également la proportion d'IgG1 / IgG3 par rapport à IgG2 / IgG4 à chaque instant et la tendance dans le temps et évaluer si le biais rapporté en IgG1 / IgG3 est maintenu dans cette importante cohorte de survivants. Pour l'analyse des données, nous utiliserons le modèle ANOVA pour les données quantitatives et des modèles linéaires généralisés pour évaluer

les relations entre les facteurs qualitatifs (séquelles post-Ebola, gravité de la maladie, genre, etc.) et les données quantitatives.

Cette étude portera sur l'ensemble de la population de la cohorte des survivants.

10.3.4. Etude immunologique cellulaire

L'analyse des données sera réalisée de la même façon que celle des données de l'ensemble de la cohorte. Une analyse descriptive des résultats de ce volet immunologique sera réalisée.

Des analyses multi variées seront réalisées pour l'analyse des différents critères de jugement permettant un ajustement sur des co-variables d'intérêt (âge, sexe, sévérité de l'infection initiale, etc.) par des modèles des risques proportionnels de Cox pour l'étude des facteurs binaires à étudier en fonction du temps. Pour les critères de jugement non dépendants du temps, des modèles logistiques, polytomiques ou linéaires seront utilisés respectivement pour des variables à expliquer binaire, catégorielle ou continues. A partir des résultats des modèles multivariés, des scores seront construits à partir des variables indépendantes et des coefficients de régression, pour donner une mesure globale de l'état physique et de l'état psychique. Un seuil de 0,20 sera utilisé pour la sélection des variables en analyse univariée.

Compte-tenu de la nature répétée des mesures réalisées, les données seront aussi analysées avec des modèles mixtes généralisés. L'hétérogénéité liée au recrutement dans la cohorte à des temps variables après sortie du CTE sera prise en compte.

Le seuil global des tests sera de 5% et les analyses seront réalisées sur les logiciels Stata 12/SE et R.

10.3.5. Etude génétique

Deux phénotypes principaux seront étudiés : la résistance à l'infection en comparant le sous-ensemble A (individus contacts non infectés) par rapport aux sous-ensembles B (individus contacts infectés) + C (survivants à la MVE) et le développement d'une maladie clinique chez les sujets infectés en comparant le sous-ensemble C par rapport au sous-ensemble B. Nous rechercherons des candidats variants rares codants (CVC, Coding Variant Candidate) ayant un fort impact (odds ratio (OR) $<0,1$ ou >10) soit sur la résistance à l'infection (comparaison du sous-ensemble A vs B + C), soit dans le développement d'une maladie clinique (sous-ensemble C vs B). Ces effets forts en termes d'OR permettent d'avoir une puissance suffisante pour les analyses qui seront menées

Cette recherche sera menée en réalisant un séquençage d'exomes chez l'ensemble des individus. Brièvement, l'analyse des variants rares provenant de données d'exomes repose sur des méthodes statistiques spécifiques, qui sont globalement basées sur l'agrégation des variants rares (par exemple avec une fréquence $< 1\%$) au sein d'une unité génétique (le gène dans un premier temps), ce qui permet de tester l'effet cumulatif de multiples variants au sein de cette unité génétique. L'approche la plus simple consiste à sommer le nombre d'allèles mineurs observés pour chaque variant du gène et à comparer cette somme entre les cas et les témoins tels que définis précédemment (par exemple les survivants du groupe C vs les contacts infectés du groupe B pour étudier le développement de la maladie clinique). Nous rechercherons les gènes pour lesquels il existe soit un fort excès (OR >10) ou un fort défaut (OR $<0,1$) en variants rares chez les cas par rapport aux contrôles. L'impact d'un variant donné peut être pondéré en fonction de sa fréquence, ou de son impact fonctionnel prédit (par exemple variants non synonymes, codons stop, sites d'épissage alternatif). Dans une seconde étape « circuit-

centré », l'unité d'étude peut être constituée par un ensemble de gènes appartenant à la même cascade biologique (Itan Y et al, PNAS 2013 110(14):5558-63). Plusieurs méthodes statistiques et logiciels dédiés ont été développés pour ces analyses (Lee S, Abecasis GR, Boehnke M, Lin X. *Am J Hum Genet* 2014; **95**(1): 5- 23). Le ou les gènes (indépendants ou appartenant à un même circuit) qui sont alors identifiés par ce criblage initial avec un certain niveau de signification (par exemple $p < 0.001$) feront alors l'objet d'une exploration approfondie avec :

- a) confirmation des variants fournis par l'exome par séquençage classique Sanger ;
- b) étude de réplication dans les cohortes de Guinée.

Les variants qui seront confirmés pourront alors faire l'objet d'études fonctionnelles ultérieures.

11. CONFIDENTIALITE

11.1. Modalités de respect de la confidentialité vis-à-vis des personnes

Avant inclusion, un numéro de screening spécifique, différent du code de suivi des participants inclus dans l'étude sera attribué.

Pour l'étude de cohorte « les vainqueurs » après consentement du participant, un numéro d'identification unique lui sera attribué (ID patient). Il sera constitué du code du centre d'inclusion (1 chiffre) suivi d'un numéro d'ordre incrémenté (3 chiffres) et d'une clef de vérification unique (code à 4 lettres).

Pour l'étude des contacts après consentement, un numéro d'identification unique lui sera attribué (ID contact). Il sera constitué d'un numéro d'unité domestique (code incrémenté à 3 chiffres) et d'un numéro d'ordre (code à 4 chiffres).

Des étiquettes code QR seront utilisées sur les différents documents de l'étude et sur les échantillons biologiques.

Toute personne ayant accès aux données, y compris les investigateurs principaux, est soumise au secret professionnel. Durant l'étude ou à son issue, les données recueillies sur les personnes qui s'y prêtent et transmises au promoteur par les investigateurs (ou tous autres intervenants spécialisés) seront pseudonymisées grâce au numéro d'identification unique. Elles ne doivent en aucun cas faire apparaître en clair les noms des personnes concernées ni leur adresse. Le promoteur et les investigateurs s'assureront que chaque personne qui se prête à la recherche a donné son accord par écrit pour l'accès aux données individuelles la concernant et strictement nécessaires au contrôle de qualité de la recherche.

11.2. Modalités de respect de la confidentialité vis-à-vis de la recherche

Les données générées lors de cette étude sont confidentielles et disponibles sur demande en cas d'audit du promoteur ou d'inspection des autorités administratives compétentes. Toute personne bénéficiant d'un accès aux données est assujettie au secret professionnel.

12. ASPECTS ETHIQUES

12.1. Justification éthique de la recherche

Ce projet apportera un bénéfice direct aux survivants à travers une amélioration du diagnostic et de la prise en charge gratuite de leurs séquelles.

12.2. Adéquation du lieu à la recherche

Les participants sont inclus à l'hôpital et les prélèvements pour la recherche sont réalisés dans le cadre de leur suivi médical, les conditions relatives aux moyens humains, matériels et techniques sont assurées. Si un incident devait se produire, les patients bénéficieraient de tous les moyens humains et techniques de l'hôpital pour garantir leur sécurité conformément aux règles d'hygiène et de sécurité en vigueur et dans le respect de l'intégrité du patient.

12.3. Dispositions éthiques et réglementaires

Cette étude est réalisée en respect des principes éthiques énoncés dans la version révisée actuelle de la Déclaration d'Helsinki (64^{ème} Assemblée Générale de l'Association Médicale Mondiale, Fortaleza, Brésil, octobre 2013), des recommandations des Bonnes Pratiques Cliniques – ICH (1996), les lignes directrices éthiques internationales CIOMS/OMS pour la recherche biomédicale impliquant des sujets humains (1993, révisées en 2002) et conformément à la législation nationale de la République Démocratique du Congo.

Le protocole sera soumis au Comité d'Ethique pour la Recherche de l'Ecole de Santé Publique de Kinshasa, RDC et au comité d'évaluation éthique de l'Inserm pour avis et approbation.

Avant transmission de toute donnée en France, une demande d'autorisation de la recherche sera faite à la Commission Nationale de l'Informatique et de Libertés (CNIL) dans le respect de la loi n°78-17 relative à l'Informatique, aux fichiers et aux Libertés du 6 janvier 1978 modifiée notamment par la loi 2004-801 du 6 août 2004 et le décret d'application n°2005-1309 du 20 octobre 2005, complété par la décision du 5 janvier 2006 portant homologation d'une méthodologie de référence, MR-001, pour les traitements de données personnelles opérés dans le cadre des recherches biomédicales. Une lettre d'information sera donnée à chaque participant et un consentement écrit sera demandé à tous les sujets participants.

Le transfert éventuel des échantillons vers la France ne se fera qu'après réception de l'autorisation d'« importation d'éléments du corps humain à des fins d'études scientifiques » conformément aux articles R1235-7 et R1235-8 du code de la santé publique français.

La version définitive du protocole sera approuvée par le promoteur, les investigateurs et co-investigateurs principaux.

L'étude sera conduite conformément au présent protocole. Hormis dans les situations d'urgence nécessitant la mise en place d'actes thérapeutiques spécifiques, les investigateurs ou leurs représentants désignés s'engagent à respecter le protocole en tous points, en particulier en ce qui concerne le recueil du consentement libre et éclairé.

Un rapport annuel de l'étude sera rédigé et adressé aux autorités éthiques et réglementaires. Une autorisation des différents lieux de recherche sera requise avant le début de cette étude.

12.4. Assurances et financements

L'Inserm, en tant que promoteur, a souscrit pour toute la durée de la recherche un contrat d'assurance en responsabilité civile sous le numéro 12001 – 09005/1001/145 0000431.

13. REGLES DE PUBLICATIONS

En respect des engagements pris par l'Inserm et des obligations réglementaires, cette recherche promue par l'Inserm sera enregistrée sur le site public www.clinicaltrials.gov.

13.1. Modalités relatives à la publication des résultats

Toutes les données recueillies au cours de cette recherche sont la propriété du promoteur et ne peuvent être communiquées en aucun cas à une tierce personne sans l'accord écrit du promoteur.

Les résultats sont publiés après analyse finale sous la forme d'articles scientifiques dans des revues à comité de lecture, exposés lors de conférences nationales et internationales. Toute publication ou communication (orale ou écrite) est décidée d'un commun accord entre les investigateurs coordonnateur et principaux, le responsable scientifiques, le promoteur et respectera les recommandations internationales: "Uniforms Requirements for

Manuscripts Submitted to Biomedical Journals"

(<http://www.cma.ca/publications/mwc/uniform.htm>).

Toute publication doit suivre les règles présentes dans la charte des publications définie par AVIESAN. La mention de l'origine du financement, des autorisations des autorités compétentes, du consentement des participants doit apparaître dans les remerciements selon le modèle suggéré ci-dessous :

*/Ethics statement */This study is part of clinical trial ****CXX-XX sponsored by Inserm. It was granted approval by local Ethics Committee (XXX) on ---****DATE***, and registered in a public trials registry (****CT XXXX**).All study participants gave their informed, written consent to participation, in line with French legal guidelines. En respect des engagements pris par l'Inserm et des obligations réglementaires les résultats seront publiés sur le site public sur lequel aura été enregistré l'essai.

13.2. Modalités d'informations des personnes ayant participé à la recherche sur ses résultats globaux

A l'issue de la recherche, la personne qui s'y est prêtée a le droit d'être informée des résultats globaux de cette recherche, selon les modalités qui lui seront décrites.

Les participants à la recherche seront invités après publication des résultats afin de les informer de ceux-ci.

13.3. Modalités d'information des personnes sur les données de santé en cours et après la recherche

Les personnes pourront à tout moment être informées des données de santé les concernant, sur simple demande.

Toute anomalie cliniquement significative détectée dans les résultats d'examen ou d'analyse sera communiquée au participant et au médecin préalablement choisi par le participant sauf si ce dernier s'y est opposé.

En cas d'études des caractères génétiques, un soin particulier doit être apporté à la rédaction des dispositions concernant les découvertes dites incidentales ou secondaires dans la mesure où les modalités de leur divulgation doivent être très précisément décrites.

La personne dont la participation est sollicitée est informée de son droit d'avoir communication, au cours ou à l'issue de la recherche, des informations concernant sa santé, détenues par l'investigateur ou, le cas échéant, le médecin ou la personne qualifiée qui le représente.

A titre exceptionnel, lorsque dans l'intérêt d'une personne malade le diagnostic de sa maladie n'a pu lui être révélé, l'investigateur peut, dans le respect de sa confiance, réserver certaines informations liées à ce diagnostic.

Aucune information à caractère médical ne sera délivrée à un participant par un personnel de la recherche non médecin.

13.4. Modalités relatives à la communication presse

Les articles et résumés mais également les communications orales issus de cette étude seront adressés avant publication au Pôle de Recherche Clinique et au Département de l'information scientifique et de la communication (DISC).

14. GOUVERNANCE ET COMITE

Dans le cadre de la mise en place de cette étude, plusieurs structures sont impliquées. Ce sont :

L'Institut National de Recherche Biomédicale (INRB)Le

Programme National de Suivi des Guéris (PNSG)

Les services de prise en charge des guéris des hôpitaux de Beni, investigateur principal (IP) : Dr John Kakule Manzombo, Butembo, IP : Dr Skoda Mumbere Muyisa , Mambasa, IP : Dr Béatrice Dekolo Osakoet Mangina, IP : Dr Serge Mumbere Kavalami.

TransVIHMI, Inserm U1175, IRD UMI233, Université de Montpellier.

Vaccine Research Institute, Hôpital Henri Mondor, Inserm U955, Créteil, France.

Inserm/Laboratoire de génétique humaine des maladies infectieuses, Institut Imagine, Inserm U1163, Paris, France

Inserm/Pôle de recherche clinique

Inserm/Institut thématique d'immunologie, inflammation, infectiologie et microbiologie

14.1. Coordination de l'étude

14.1.1. Les investigateurs principaux

Les investigateurs principaux assurent :

- la responsabilité globale de l'étude,

- la revue du protocole,
- la formation et la coordination des investigateurs associés,
- la revue de toutes les procédures spécifiques à l'étude,
- l'archivage des documents de l'étude,
- le suivi général de l'étude,
- la gestion du budget de l'étude et la trésorerie,
- la rédaction du rapport final.

14.1.2. Les co-investigateurs et investigateurs associés :

Les co-investigateurs et investigateurs associés supervisent:

- le screening des sujets de l'étude,
- le recueil du consentement éclairé,
- l'inclusion des patients,
- le suivi médical et biologique des patients selon le protocole de recherche approuvé par le comité d'éthique pour la recherche de l'école de Santé Publique de Kinshasa,
- la remise des résultats aux patients,
- le suivi et la prise en charge des événements cliniques,
- le remplissage et la bonne tenue des supports papiers (documents sources et cahiers d'observation) et électronique (base de données).

Ils seront soutenus dans ce travail par des missions régulières sur le terrain des équipes de TransVIHMI et qui auront pour mission de superviser la conduite de la cohorte sur les différents sites alternés et le lien avec le Centre de Méthodologie et de Gestion de Montpellier.

14.1.3. Les équipes associées

Analyses immuno-virologique et biothèque :

L'INRB assure :

- l'élaboration des procédures de laboratoire,
- la réalisation des examens immuno-virologiques,
- la préparation des aliquots pour la constitution de la biothèque,
- le rendu des résultats aux médecins investigateurs,
- l'archivage des documents laboratoire de l'étude.

TransVIHMI assure l'expertise virologique, l'assurance qualité et le transfert de compétences pour certains examens complémentaires (détection d'EBOV dans les fèces, l'urine, la salive et la mise au point d'un test sérologique utilisant des protéines recombinantes en Elisa ou Luminex).

Le **Vaccine Research Institute, Inserm U955** assure l'expertise immunologique.

Le laboratoire de génétique humaine des maladies infectieuses, Inserm assure l'expertise génétique.

14.1.4. L'association Nationale des Vainqueurs d'Ebola

Cette association sera directement impliquée dans le projet dans le cadre du suivi des guéris.

14.1.5. Centre de méthodologie et de gestion

Le Centre de Méthodologie et de Gestion (CMG) est situé au niveau de TransVIHMI à Montpellier. Il participera à la création d'un CMG à l'INRB dans le cadre du renforcement des compétences de ce projet. Il est mandaté par le promoteur pour

- la gestion et la coordination générale de l'étude,
- l'élaboration du protocole de l'étude et du plan d'analyse,
- la revue de toutes les procédures opérationnelles,
- l'élaboration du cahier d'observation et du masque de saisie de la base de données,
- les formations du personnel au protocole et aux procédures de l'étude,
- le monitoring de l'étude,
- le contrôle et la validation des données,
- l'expertise épidémiologique et l'analyse statistique des données,
- la rédaction du rapport final.

14.2. Le promoteur

Le promoteur assure la responsabilité légale et financière de l'étude.

14.3. Comité de suivi de l'étude

Le Comité de suivi est constitué des représentants des partenaires locaux de l'étude : Investigateur principal, chef de projet, investigateurs associés (biologique et clinique), et deux représentants du centre de méthodologie et de gestion. Il se réunit aux différentes étapes de l'étude (suivi des inclusions, prise en charge des événements cliniques et gestion des ressources humaines et financières de l'étude, etc...).

14.4. Conseil scientifique de l'étude

Le Conseil scientifique (CS) se réunit à toutes les étapes majeures de l'étude : à la fin des inclusions, puis au moins une fois par an au cours du déroulement de l'étude, à la présentation successive des résultats, et à la fin de l'étude. Il valide la version du protocole à déposer au comité d'éthique et le cas échéant les amendements au protocole.

Le CS de l'étude a pour mission de veiller au bon déroulement de la recherche, tant sur le plan scientifique, qu'éthique et logistique, et d'en répondre, notamment vis-à-vis du promoteur.

- Il s'assure régulièrement de la bonne marche de l'étude et du respect du protocole,
- Il assure le suivi scientifique de la recherche : maintien de la pertinence des questions de recherche et de la validité des méthodes mises en œuvre pour y répondre,
- Il veille à l'application des règles d'accès aux données de la recherche et de communication et publication de ses résultats,
- Il maintient un lien permanent avec le promoteur et les investigateurs,

- Il décide de toute modification pertinente du protocole nécessaire à la poursuite de la recherche, notamment :
 - o Les mesures permettant de faciliter le recrutement dans la recherche,
 - o Les amendements au protocole avant leur présentation au comité d'éthique
 - o Les décisions d'ouvrir ou de fermer un site participant à la recherche.

Après chaque réunion un compte-rendu de séance est rédigé par le chef de projet en collaboration avec le président du CS. Le compte rendu est diffusé aux membres du CS, aux personnes invitées lors de la réunion et au représentant du promoteur.

15. ASSURANCE QUALITE

15.1. Description

Le rôle de l'assurance qualité est de garantir la sécurité des personnes qui se prêtent aux recherches impliquant la personne humaine et d'assurer la crédibilité des données issues de ces recherches et leur reconnaissance par la communauté médicale et scientifique.

L'investigateur est le garant de la qualité du déroulement de la recherche. Elle est encadrée selon les procédures standards de l'Inserm et les procédures spécifiques à la recherche le cas échéant. Toutes les procédures spécifiques de la recherche doivent être validées par le promoteur.

15.2. Monitoring (contrôle qualité de la recherche)

Un plan de monitoring est établi par le promoteur, validé par l'investigateur et le cas échéant le responsable de la gestion des données. Il précise les modalités d'ouverture, de visite et de clôture des centres investigateurs ainsi que les modalités de surveillance de la recherche. Il tient compte de l'évaluation du risque de la recherche par le promoteur.

Les représentants du promoteur effectueront des visites dans le centre investigateur en fonction du rythme des inclusions et du plan de monitoring.

Le data manager vérifiera les consentements dont la non opposition à l'étude génétique et à la conservation des échantillons biologiques.

16. MODIFICATIONS SUBSTANTIELLES DU PROTOCOLE

Plusieurs conditions peuvent requérir une modification substantielle du protocole, notamment la prolongation de la durée du suivi.

Toute demande de modification de la recherche par rapport au projet de recherche initialement autorisé doit être soumise, par l'investigateur coordonnateur, pour avis, au promoteur.

Les modalités de soumission sont disponibles sur l'intranet de l'Inserm.

Après avis favorable, l'Inserm mettra en œuvre les procédures administratives réglementaires nécessaires à l'obtention de l'approbation de ces modifications substantielles par le Comité d'Ethique pour la Recherche de l'Ecole de Santé Publique de Kinshasa et au comité d'évaluation éthique de l'Inserm.

17. ANNEXES

1. Malvy D, et al J. Ebola virus disease. *Lancet*, 2019 ; 39: 936-948. Review
2. Goldstein, T., et al., The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. *Nat Microbiol*, 2018. 3(10): p. 1084-1089
3. World Health Organization. 2016 Situation report: Ebola virus disease, 10 June 2016. Geneva, Switzerland: World Health Organization
4. Mbala-Kingebeni P, et al. 2018 Ebola virus disease outbreak in Équateur Province, Democratic Republic of the Congo: a retrospective genomic characterisation. *Lancet Infect Dis*. 2019 ; 19 : 641-647.
5. Mbala-Kingebeni P, et al. Medical countermeasures during the 2018 Ebola virus disease outbreak in the North Kivu and Ituri Provinces of the Democratic Republic of the Congo: a rapid genomic assessment. *Lancet Infect Dis*. 2019 ; 19 : 648-657
6. Lefebvre A, et al: Case fatality rates of Ebola virus diseases: A meta-analysis of World Health Organization data. *Médecine et maladies infectieuses* 2014, 44(9):412–416.
7. Leroy, E.M., et al., Human asymptomatic Ebola infection and strong inflammatory response. *Lancet*, 2000. 355(9222): p. 2210-5.
8. Glynn, J.R., et al., Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-sectional study using a new non-invasive assay for antibodies to Ebola virus. *Lancet Infect Dis*, 2017. 17(6): p. 645-653.
9. Diallo, M.S.K., et al., Prevalence of infection among asymptomatic and paucisymptomatic contact persons exposed to Ebola virus in Guinea: a retrospective, cross-sectional observational study. *Lancet Infect Dis*, 2019. 19(3): p. 308-316.
10. PREVAIL III Study Group. A Longitudinal Study of Ebola Sequelae in Liberia. *N Engl J Med*, 2019 ; 380 : 924-934
11. Etard JF, et al. Multidisciplinary assessment of post-Ebola sequelae in Guinea (Postebogui): an observational cohort study. *Lancet Infect Dis*, 2017; 17 :545-552
12. Bwaka MA, et al I: Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. *J Infect Dis* 1999, 179 Suppl 1:S1-7.
13. Kibadi K, et al : Late ophthalmologic manifestations in survivors of the 1995 Ebola virus epidemic in Kikwit, Democratic Republic of the Congo. *J Infect Dis* 1999, 179 Suppl 1:S13-14.
14. Keita A et al, Long-term follow-up of Ebola virus in body fluids from Ebola virus disease survivors in Guinea (Postebogui) reveals longterm persistence in semen and breast milk. *Soumis*
15. Ayouba, A., et al., Development of a Sensitive and Specific Serological Assay Based on Luminex Technology for Detection of Antibodies to Zaire Ebola Virus. *J Clin Microbiol*, 2017. 55(1): p. 165-176.
16. Sobarzo, A., et al., Profile and persistence of the virus-specific neutralizing humoral immune response in human survivors of Sudan ebolavirus (Gulu). *J Infect Dis*, 2013. 208(2): p. 299-309.
17. Rimoin, A.W., et al., Ebola Virus Neutralizing Antibodies Detectable in Survivors of the Yambuku, Zaire Outbreak 40 Years after Infection. *J Infect Dis*, 2018. 217(2): p. 223-231.
18. Davis CW, et al. Longitudinal Analysis of the Human B Cell Response to Ebola Virus Infection. *Cell*. 2019 May 30;177(6):1566-1582.e17.
19. Gunn BM, et al. Survivors of Ebola virus disease develop polyfunctional antibody responses. *J Infect Dis*. 2019 Jul 12. pii: jiz364. doi: 10.1093/infdis/jiz364.
20. Corti, D., et al., Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody. *Science*, 2016. 351(6279): p. 1339-42
21. Lubaki NM, et al The Lack of Maturation of Ebola Virus-Infected Dendritic Cells Results from the Cooperative Effect of at Least Two Viral Domains. *J. Virol* 2013. 87(13): p7471-85
22. Basler, C., et al The Ebola virus VP35 protein functions as a type I IFN antagonist. *Proc Natl Acad Sci U S A*, 2000. 97(22): p.12289-94.
23. Baize, S., et al . (2002). Inflammatory responses in Ebola virus- infected patients. *Clin. Exp. Immunol*. 128, 163–168.
24. Wiedemann A et al,; PostEboGui Study Group. Long-lasting Ebola-specific T cell immunity in Guinean Ebola survivors. Poster presentation ECCMID, Amsterdam April 2019
25. Alcais A, et al: Human genetics of infectious diseases: between proof of principle and paradigm. *J Clin Invest* 2009, 119(9):2506-2514.
26. Rasmussen, A.L., Host Factors in Ebola Infection. *Annu Rev Genomics Hum Genet*, 2016. 17: p. 333-51.
27. Rasmussen, A.L., et al., Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance. *Science*, 2014. 346(6212): p. 987-91.

28. Kuroda, M., et al., Interaction between TIM-1 and NPC1 Is Important for Cellular Entry of Ebola Virus. *J Virol*, 2015. 89(12): p. 6481-93.
29. Carette, J.E., et al., Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature*, 2011. 477(7364): p. 340-3.
30. Cote, M., et al., Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. *Nature*, 2011. 477(7364): p. 344-8.
31. Herbert, A.S., et al., Niemann-pick c1 is essential for ebolavirus replication and pathogenesis in vivo. *MBio*, 2015. 6(3).
32. Ng, M., et al., Filovirus receptor NPC1 contributes to species-specific patterns of ebolavirus susceptibility in bats. *Elife*, 2015. 4.
33. Kondoh, T., et al., Single-Nucleotide Polymorphisms in Human NPC1 Influence Filovirus Entry Into Cells. *J Infect Dis*, 2018.
34. Sakurai, Y., et al., Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment. *Science*, 2015. 347(6225): p. 995-8.
35. Batra, J., et al., Protein Interaction Mapping Identifies RBBP6 as a Negative Regulator of Ebola Virus Replication. *Cell*, 2018. 175(7): p. 1917-1930 e13.
36. Shtanko, O., et al., Autophagy-Associated Proteins Control Ebola Virus Internalization Into Host Cells. *J Infect Dis*, 2018. 218(suppl_5): p. S346-S354.
37. Le Marcis F et al (to be published in September 2019) « Three Acts of Resistance during the 2014– 2016 West Africa Ebola Epidemic: A Focus on Community Engagement. » *Journal of Humanitarian Affairs*. Vol 1(2): 23–31. <http://dx.doi.org/10.7227/JHA.004>
38. Bamshad, M.J., et al., Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet*, 2011. 12(11): p. 745-55.
39. Belkadi, A., et al., Whole-exome sequencing to analyze population structure, parental inbreeding, and familial linkage. *Proc Natl Acad Sci U S A*, 2016. 113(24): p. 6713-8.
40. Li, H. and R. Durbin, Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*, 2010. 26(5): p. 589-95.
41. Boisson-Dupuis, S., et al., Tuberculosis and impaired IL-23-dependent IFN-gamma immunity in humans homozygous for a common TYK2 missense variant. *Sci Immunol*, 2018. 3(30).
42. Meyts, I., et al., Exome and genome sequencing for inborn errors of immunity. *J Allergy Clin Immunol*, 2016. 138(4): p. 957-969.
43. Okada, S., et al., Impairment of immunity to *Candida* and *Mycobacterium* in humans with bi-allelic RORC mutations. *Science*, 2015. 349(6248): p. 606-13.

Annex 2

Dynamique de l'épidémie à SARS-CoV-2 à Kinshasa, République Démocratique du Congo

Etude ARIACOV

Protocole version 1.0 du 10 Septembre 2020

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LISTE DES ABREVIATIONS

AFD	Agence Française de Développement
ARIACOV	Appui à la Riposte africaine à l'épidémie COVID-19
AS	Aire de Santé
COVID-19	Maladie causée par le coronavirus SARS-Cov-2
IRD	Institut de Recherche pour le Développement
INRB	Institut National de recherche Biomédicale
OMS	Organisation Mondiale de la Santé
PCR	Polymerase chain reaction
RDC	République Démocratique du Congo
SARS-COV2	Severe Acute Respiratory Syndrome – Coronavirus 2
SOP	Procédure Opérationnelle Standardisée
TransVIHMI	Recherches translationnelles sur le VIH et les maladies infectieuses
UMI	Unité mixte internationale
ZS	Zone de Santé

RESUME

Protocole version 1.0 du 10 Septembre 2020

Dynamique de l'épidémie à SARS-CoV-2 à Kinshasa, République Démocratique du Congo

ARIACOV : Appui à la Riposte Africaine à l'épidémie de COVID-19

Pays participant : République Démocratique du Congo

Objectifs

Objectif principal

L'objectif principal de l'étude est de décrire la dynamique de l'épidémie à SARS-CoV-2 dans la ville de Kinshasa, représentée par l'évolution de la séroprévalence au cours du temps, stratifiée par zone géographique (Est-Ouest).

Objectifs secondaires

1. Estimer la séroprévalence globale, par classe d'âge dans chacune des enquêtes
2. Estimer la proportion de cas asymptomatiques ou pauci-symptomatiques parmi les participants avec une sérologie positive à l'infection au virus SARS-CoV-2
3. Estimer dans chaque enquête le nombre de cas confirmés par PCR ou probables d'infection à SARS-CoV-2 parmi les participants présentant une sérologie positive ou négative.
4. Analyser la relation entre les caractéristiques démographiques et cliniques, un éventuel contact avec un cas confirmé ou probable et le statut sérologique vis-à-vis du virus SARS-CoV-2.

Méthodologie

Trois enquêtes répétées en population générale espacées de 6 semaines entre elles. Pour chaque enquête, un plan d'échantillonnage en grappes à deux degrés sera utilisé après stratification du site d'étude en 2 zones géographiques : Est et Ouest de la ville de Kinshasa. Le but est d'obtenir un échantillon représentatif de la population de Kinshasa. Chaque enquête est indépendante l'une de l'autre.

Pour ce faire, l'étude se déroulera dans 14 zones de santé (ZS) à raison de 7 ZS dans chacune des 2 strates (Est et Ouest). Chaque zone de santé sera considérée comme un domaine d'étude. Les aires de santé (AS) vont constituer des grappes. L'unité de sondage primaire sera la grappe, en l'occurrence l'aire de santé. Au total 3 AS seront tirées aléatoirement par zone de santé.

Nombre prévu de participants : 1 240 individus par enquête

La taille minimale est calculée en tenant compte des considérations suivantes : une différence de séroprévalence dans la population générale de 10% entre la première et la deuxième enquête ; en supposant que la séroprévalence attendue pour la première enquête soit de 10%, si nous voulons estimer cette séroprévalence au niveau provincial avec une marge d'erreur de 5% , un coefficient de corrélation intra-grappe de 0,05 et une proportion des non-réponses de 20%, la taille minimale de l'échantillon sera de 1 020 sujets pour chaque enquête.

En prenant l'hypothèse d'un taux de participation de 80%, d'une moyenne de 5 individus par ménage visité (en moyenne 5 résidents par ménage en RDC, EDS 2013-2014), il faudra inclure dans chaque enquête 1 240 sujets, soit 90 sujets par zone de santé et 30 sujets par aire de santé répartis dans 8 ménages.

Estimant que 50% de la population est âgé de plus de 18 ans, dans 50% des ménages, tous les résidents seront invités à participer à l'étude et dans les 50% autres, seuls les individus de 18 ans et plus seront invités à participer.

Critères de jugement

- **Critère de jugement principal** : La différence de séroprévalence entre deux enquêtes, stratifiée par zone géographiques Est et Ouest ?
- **Critères de jugement secondaires** :
 - Séroprévalence définie comme la proportion de participants par classe d'âge présentant des anticorps IgG anti- SARS-CoV-2.
 - La proportion de cas symptomatiques définie comme la présence de symptômes de l'infection au virus SARS-CoV-2 depuis le début de l'année parmi les participants avec une sérologie SARS-CoV-2 positive :
 - Fièvre
 - Fatigue
 - Toux (sèche)
 - Rhinite
 - Mal de gorge
 - Difficulté à respirer
 - Douleurs thoraciques
 - Douleurs musculaires
 - Nausée/Vomissement
 - Signes dermatologiques, anosmie, dysgueusie
 - Asthénie
 - Anorexie
 - La proportion de cas asymptomatiques parmi les participants avec une sérologie SARS-CoV-2 positive.
 - Le nombre de cas antérieurement confirmés par PCR.
 - Le nombre de cas antérieurement classés probables ([https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-\(2019-ncov\)](https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov))).
 - Le contact avec un patient suspect ou confirmé d'infection au virus SARS-CoV-2.
 - La proportion d'individus qui sont allés consulter un prestataire de soins de santé pour un ou plusieurs de ces symptômes.
 - Parmi les individus avec une sérologie positive, la proportion qui a été diagnostiquée infection à SARS-CoV-2 et confirmée par test PCR, la proportion qui a été diagnostiquée infection probable et la proportion qui n'a pas été diagnostiquée.

Population d'étude

- **Critères d'inclusion** :
 - **Pour les participants adultes (âge ≥ 18 ans)** : Accepter de participer à l'étude et signer le consentement avant toute intervention

- **Pour les participants mineurs (âge entre 10 ans et 17 ans):** consentement éclairé signé par au moins un des deux parents ou le tuteur légal autorisant la participation de l'enfant à l'étude ainsi que l'assentiment écrit de l'enfant pour participer à l'étude.
- **Pour les participants mineurs (âge < 10 ans):** consentement éclairé signé par au moins un des deux parents ou le tuteur légal autorisant la participation de l'enfant à l'étude.
- **Critères de non-inclusion :**
 - Impossibilité de se conformer aux exigences et procédures de l'étude selon l'opinion de l'investigateur ;
 - Incapacité de consentir pour les adultes et enfants de plus de 10 ans.

Méthodes d'analyse statistique :

Le questionnaire ménage sera utilisé pour des analyses descriptives sur la population incluse et ceux qui n'ont pas participé à l'étude sérologique.

Pour chaque participant, plusieurs dimensions seront considérées. Les analyses descriptives seront pondérées pour prendre en compte la probabilité de sélection de l'échantillonnage en grappe et seront présentées avec un intervalle de confiance à 95%. Les statistiques du test t simples et du chi² de Pearson seront utilisées pour comparer les résultats descriptifs continus et catégoriels, respectivement. Les résultats seront calculés avec les intervalles de confiance correspondants à 95 % et stratifiés par groupe d'âge, sexe et zone géographique (Est-Ouest). Le coefficient de corrélation intra-famille effectif sera calculé à l'issue de la première enquête.

Les résultats sérologiques seront stratifiés par âge, zone géographique et pondérés pour prendre en compte l'effet lié à l'échantillonnage. Des modèles logistiques multivariés évalueront l'association entre la séroprévalence et les facteurs de risque dans chaque étude. Après la première enquête, une analyse classique transversale sera conduite suivie d'une approche incrémentielle en poolant les données issues des enquêtes ultérieures et en introduisant une covariable codant pour la prévalence à chaque enquête. L'hétérogénéité spatiale sera prise en compte.

La dynamique de l'épidémie sera modélisée en utilisant des modèles linéaires généralisés. De plus, les modèles de diffusion de l'épidémie qui seront disponibles, publiés par d'autres équipes, seront appliqués aux données afin d'estimer les paramètres de diffusion observés à Kinshasa (incidence cumulée, temps de doublement, variabilité spatiale de la transmission).

Calendrier/Echéancier prévisionnel

Date de début de la recherche : Octobre 2020

Durée des inclusions : 4 mois

Durée de participation par participant : Une journée

Durée totale prévue de la recherche : 12 mois

Date prévisionnelle de la fin de la recherche : Septembre 2021

I. RATIONNEL SCIENTIFIQUE ET JUSTIFICATION DE LA RECHERCHE

1. Contexte

1.1. Généralité sur le COVID-19

La détection et la propagation d'un agent pathogène respiratoire émergent s'accompagnent d'une incertitude quant à ses principales caractéristiques épidémiologiques et sérologiques, et notamment sa capacité à se propager dans la population humaine.

C'est le cas du virus SARS-CoV-2, détecté pour la première fois dans la ville de Wuhan, en Chine, en décembre 2019(1). Très rapidement, dans chaque pays touché par la pandémie, les capacités diagnostiques initiales ont été dépassées et la surveillance s'est principalement concentrée sur les patients atteints de formes sévères nécessitant une hospitalisation et les personnels de santé.

Le nombre de patients qui sont ou ont été infectés par la maladie est donc inconnu. En outre, les informations sur la proportion d'infections peu ou pas symptomatiques et leur rôle dans la transmission interhumaine sont incomplètes et discordantes. Alors que le rapport de l'OMS après sa visite initiale en Chine évoquait peu de cas asymptomatiques, les premiers résultats d'une étude italienne où la population entière d'un village a été dépistée par PCR pour le SARS-CoV-2 a montré que la moitié des patients infectés ne montraient pas de symptômes au moment du test (2,3). Nous ne savons pas si ces patients étaient asymptomatiques ou pré symptomatiques. Dans une autre étude effectuée dans une maison de retraite, 10 des 13 patients asymptomatiques au moment du test ont développé des symptômes après 7 jours d'observation, mais l'âge moyen de ces patients était de 80 ans, rendant difficile la généralisation de ces résultats à une population plus jeune (4).

La dynamique de la pandémie et l'impact des interventions sont aujourd'hui exclusivement simulés par des modèles qui pourraient être améliorés par de meilleures données observées en population informant sur le niveau d'immunité de groupe. C'est dans ce cadre que l'Organisation Mondiale de la Santé (OMS), recommande la réalisation d'études répétées de séroprévalence en population afin de mesurer la dynamique de l'épidémie (5). Ces études sont d'autant plus importantes en Afrique sub-Saharienne où l'accès aux soins et aux tests diagnostic PCR est limité et où la population, plus jeune, pourrait avoir plus de chance d'être asymptomatique en cas d'infection.

1.2. Epidémiologie de la COVID-19 à Kinshasa

En République Démocratique du Congo, le premier cas de COVID-19 a été détecté le 10 mars à Kinshasa. Il s'agit d'un patient de nationalité congolaise résidant en France qui est arrivé à Kinshasa le 08 mars pour une visite familiale. D'autres cas importés ont été rapportés jusqu'au 18 mars où on a rapporté les premiers cas issus d'une transmission locale. Depuis, on assiste à une augmentation progressive des cas telle que rapporté par le Ministère de la santé atteignant en date du 09 Août 2020, 9 488 cas confirmés dont 7 768 cas à Kinshasa et 224 décès.

Kinshasa est de loin, la province la plus touchée avec 81% des cas notifiés dans tout le pays en date du 09 Août 2020. Les zones de santé les plus affectées sont Limete (671 cas), Gombe (669 cas) et Binza Ozone (608 cas), qui sont respectivement situées à l'Est, au Nord-Ouest et à l'Ouest de la ville de Kinshasa. Le 26 Avril, un premier cas a été confirmé en milieu carcéral dans la ville de Kinshasa. Entre le 25 Avril et le 03 Mai, 100 nouveaux cas confirmés ont été notifiés dans la même prison, ce qui devrait être un facteur de propagation à grande échelle dans la province de Kinshasa. Kinshasa se caractérise par une très forte densité, avec 577 habitants au km², représentant ainsi plus de 34% de la population urbaine de la RDC. Aussi, la densité de la population est plus élevée du côté Est de la ville, atteignant près de 700 habitants au Km². Cependant plus de cas positifs au SARS-CoV 2 ont été diagnostiqués dans les zones de santé situées du côté Ouest et Nord-Ouest de la ville, comparé à celles situées du côté Est. Cette observation a

été faite malgré le fait que la densité de la population, la promiscuité et la faible observance des mesures barrières observées du côté Est devraient plutôt favoriser l'explosion de plus de cas dans cette partie de la ville.

1.3. Riposte à l'épidémie de la COVID-19 en République Démocratique du Congo

Une coordination de la riposte contre la COVID-19 a été mise en place avec l'Institut National de recherche Biomédicale (INRB) comme seul laboratoire habilité à poser le diagnostic de la maladie dans un premier temps jusqu'à la décentralisation du diagnostic en début Juin 2020. Ce diagnostic est réalisé par la recherche de l'ARN viral (test PCR) chez des patients symptomatiques, répondant à la définition de cas édicté par le Ministère de santé de la RDC. Cependant, une réponse calibrée à l'épidémie doit tenir compte du nombre de cas dans la population générale, y compris chez les personnes asymptomatiques infectées, dont la plupart ne sont pas détectés par le système de détection (épidémiologique et biologique) mis en place (6,7). De plus, il a été constaté que la majorité de tests étaient réalisés sur des échantillons prélevés chez les suspects provenant de zones de santé situées à l'Ouest de la ville. En revanche, très peu de cas suspects ont été rapportés dans les zones de santé de l'Est de la ville. Par conséquent, la surveillance de la séropositivité aux anticorps dans la population peut permettre de tirer des conclusions sur l'étendue de l'infection et sur l'incidence cumulée de l'infection dans la population.

2 Implications des équipes

La République Démocratique du Congo est confrontée à une recrudescence des épidémies dues au virus Ebola depuis sa découverte en 1976 à Yambuku. La 10^{ème} qui a sévi dans les provinces du Nord et Sud Kivu a été la plus importante en termes de durée ainsi que du nombre des cas et décès. L'Institut National de Recherche Biomédicale (INRB) a été à l'avant-poste de la riposte et dirige par le biais du secrétariat technique, la présente épidémie du Corona virus et la 11^{ème} épidémie d'Ebola à l'ancienne province de l'Equateur. L'INRB joue un rôle central dans la riposte grâce au renforcement de ses compétences en virologie et cela avec l'appui du Laboratoire Mixte International (LMI) qui associe TransVIHMI à Montpellier et le Centre de Recherches sur les Maladies Emergentes, Ré-émergentes et la médecine nucléaire (CREMER) de Yaoundé, Cameroun.

Depuis la déclaration de l'épidémie de Corona virus en RDC le 10 Mars 2020, le Professeur Muyembe a été nommé secrétaire technique à la riposte et le Professeur Ahuka Incident Manager. Le docteur Nkwembe est à la tête de la « commission laboratoire » et dirige le laboratoire national de surveillance des maladies respiratoires. Le laboratoire de sérologie a été équipé en matériel de Luminex et trois agents ont bénéficié d'une formation sur l'utilisation du Magpix et la maintenance. Leur implication dans l'étude est un gage du bon déroulement de l'étude et leur présence au sein des instances nationales de lutte contre COVID-19 apporte une garantie de la traduction des résultats de cette recherche en recommandations de Santé Publique, contribuant à la riposte en RDC.

TransVIHMI a une grande expérience du test sérologique multiplex Luminex. Technique utilisée par l'unité depuis 2008 sur Rétrovirus (VIH, SIV, STLV, SFV), sur Filovirus (Ebola et Marburg) et Arbovirus (Chikungunya, Dengue, Zika, West Nile, Usutu, O'nyong nyong), en tout plus de 20 articles ont été publiés (8,9). En effet si de très nombreux tests sérologiques sont en cours de développement, un point fondamental est la validation de leurs performances en Afrique. En effet d'une manière générale ces tests peuvent avoir une sensibilité et spécificité bien différentes compte tenu d'éventuelles réactions croisées avec des antigènes d'autres pathogènes.

Le test vis-à-vis du SARS-CoV-2 est prêt et a pour avantage d'être très sensible et très spécifique (10). Dans le cadre de la sérologie du SARS-CoV-2, le test permettra le diagnostic différentiel d'infection par le SARS-CoV, le SARS-CoV-2 et le Mers-Cov, à travers la recherche des IgG. Cette technique est opérationnelle à l'INRB.

3. Justification de l'étude

L'étendue et la dynamique de l'épidémie à Kinshasa, exemple d'une grande métropole africaine très densément peuplée, est inconnue. Etant donné cette densité de population, la promiscuité, la pauvreté, l'importance des contacts sociaux et économiques, une fois le virus introduit, le taux de reproduction de base effectif pourrait être supérieur au taux moyen mesuré en début d'épidémie en Chine et on pourrait s'attendre à une épidémie très dynamique (8-9).

Par ailleurs, la structure par âge très jeune de la population (41,3% a moins de 15 ans) pourrait générer une épidémie avec une expression clinique différente de l'Europe, marquée par des très nombreuses formes a- ou pauci-symptomatiques.

Toutefois, l'analyse des données issues de la riposte contre la Covid-19 à Kinshasa montre une proportion élevée des cas dans les zones de santé moins denses situées du côté Ouest et Nord-Ouest de la ville alors qu'on s'attendrait à plus de cas dans les zones de santé situées du côté Est de la ville où la densité de la population atteint près de 700 habitants au Km² et l'observance de la distanciation sociale et des mesures barrières est quasi inexistante.

Cette étude est conçue pour mesurer l'étendue de l'épidémie à SARS-CoV-2 et sa dynamique dans la ville de Kinshasa en République Démocratique du Congo, de façon stratifiée par zones géographiques Est-Ouest, au travers de trois enquêtes en population successives séparées de 6 semaines.

4. Caractère innovant de la recherche

Les études en population répétées de séroprévalence à l'infection au virus SARS-CoV-2 sont importantes pour décrire l'épidémie, sa dynamique et l'acquisition d'une immunité collective afin d'aider à la prise de décision en termes de politique de santé publique. Il n'existe pas encore de telles études en Afrique sub-Saharienne.

De plus, la connaissance de la dynamique de l'épidémie et de l'acquisition d'une immunité collective fournira une aide à la décision des acteurs de la santé publique.

Cette étude s'intègre dans un projet collaboratif entre l'IRD (Institut de recherche pour le développement) et des partenaires présents dans 6 pays d'Afrique de l'Ouest et du Centre pour étudier la dynamique de l'épidémie Covid-19 dans toute cette sous-région. Ainsi, le protocole proposé est issu d'un protocole « générique » qui a été adapté au contexte de la RDC.

5. Résultats et retombées attendus

Cette étude participera à l'amélioration des connaissances scientifiques tout en ayant un impact opérationnel direct sur les politiques de santé publique. Elle permettra pour la première fois d'observer la dynamique de l'infection à SARS-CoV-2 à Kinshasa, par 3 mesures successives espacées de 6 semaines. Cliniquement, elle permettra de mesurer la proportion de formes asymptomatiques, pauci-symptomatiques et symptomatiques. Elle permettra aussi d'évaluer séroprévalence Covid-19 à chaque enquête et de façon stratifiée entre les zones Est et Ouest de la ville de Kinshasa.

L'estimation de la progression d'une immunité de groupe contribuera à la prise de décision de santé publique, que ce soit concernant le besoin d'une vaccination ou de mesure de distanciation physique. Ces données observées permettront d'adapter les mesures de Santé Publique et de renseigner sur les modèles de diffusion de l'épidémie.

II OBJECTIFS

1. Objectif principal

L'objectif principal de l'étude est de décrire la dynamique de l'épidémie à SARS-CoV-2 dans la ville de Kinshasa, représentée par l'évolution de la séroprévalence au cours du temps, stratifiée par zone géographiques Est et Ouest.

2. Objectifs secondaires

1. Estimer la séroprévalence globale, par classe d'âge dans chacune des enquêtes
2. Estimer la proportion de cas asymptomatiques ou pauci-symptomatiques parmi les participants avec une sérologie positive à l'infection au virus SARS-CoV-2
3. Estimer dans chaque enquête le nombre de cas confirmés par PCR ou probables d'infection à SARS-CoV-2 parmi les participants présentant une sérologie positive ou négative.
4. Analyser la relation entre les caractéristiques démographiques et cliniques, un éventuel contact avec un cas confirmé ou probable et le statut sérologique vis-à-vis du virus SARS-CoV-2.

III. METHODOLOGIE

1. Schéma de l'étude

Cette étude est une étude transversale comportant trois enquêtes sérologiques répétées en population générale espacées de 6 semaines entre elles. La ville de Kinshasa sera divisée en 2 parties (strates) en fonction du nombre de cas diagnostiqués dans les zones de santé selon qu'elles sont situées à l'Est ou à l'Ouest de la ville.

2. Présentation du site de l'étude

L'enquête va se dérouler dans toutes les zones de santé de la ville-province de Kinshasa. La province de Kinshasa fait partie des 26 provinces de la RDC. En plus d'être une ville-province, elle est également la capitale politique du pays. Sur le plan sanitaire, elle est subdivisée en 35 zones de santé, qui elles-mêmes sont subdivisées en 380 aires de santé. Kinshasa est située à l'extrême ouest du pays sur les rives du fleuve Congo. La province couvre environ 9 965 km², avec une population estimée à plus de 10 millions d'habitants et un nombre moyen de 5 individus par ménage (EDS 2013-2014). La structure en âge de sa population est essentiellement dominée par les jeunes avec 41,25% des sujets âgés de 0-14 ans, 56,05% d'individus âgés de 15-64ans et 2,69% de plus de 64 ans. Divers rapports ont fait état d'une croissance démographique de 2,33% en 2018 avec un taux de natalité à 32,8 naissances pour 1000 habitants et un taux de mortalité à 9,4 décès pour 1 000 habitants. La ville a connu un rythme d'urbanisation très important dans certains quartiers d'affaires situés au centre de la ville, augmentant ainsi le coût de la vie et du logement. Pour échapper à la vie chère du centre-ville, une grande partie de la population établit résidence dans les quartiers périphériques, créant ainsi des conditions de promiscuité délétères. A cela, il faut ajouter des infrastructures de transports qui n'ont pas suivi le rythme du développement urbain, entraînant une forte promiscuité dans les transports en commun, aussi bien dans les taxis voitures que motos. Toutes ces conditions prédisposent ainsi la ville cosmopolite de Kinshasa à une extension massive du COVID-19.

3. Population d'étude

La population de l'étude est la population de la ville-province de Kinshasa. Les critères d'inclusion et de non-inclusion suivants seront appliqués :

- **Critères d'inclusion :**

- Faire partie d'un ménage sélectionné de manière aléatoire
- Être résidant de la province de Kinshasa depuis au moins 14 jours avant la date du prélèvement
- **Pour les participants adultes (âge \geq 18 ans) :** Accepter de participer à l'étude et signer le consentement avant toute intervention
- **Pour les participants mineurs (âge entre 10 ans et 17 ans) :** consentement éclairé signé par au moins un de deux parents ou le tuteur légal autorisant la participation de l'enfant à l'étude ainsi que l'assentiment écrit de l'enfant pour participer à l'étude.
- **Pour les participants mineurs (âge $<$ 10 ans) :** consentement éclairé signé par au moins un de deux parents ou le tuteur légal autorisant la participation de l'enfant à l'étude.

- **Critères de non-inclusion:**

Les adultes et enfants présentant l'une des caractéristiques ou conditions suivantes ne pourront pas être inclus dans l'étude :

- Impossibilité de se conformer aux exigences et procédures de l'étude selon l'opinion de l'investigateur ;
- Incapacité de consentir (sauf enfant de moins de 10 ans) ou révocation du consentement éclairé au cours de l'étude.

4. **Plan d'échantillonnage**

a. Taille de l'échantillon

La taille de l'échantillon a été estimée sur base de l'hypothèse de mise en évidence d'une différence de séroprévalence de 10% entre la première et la seconde enquête ; la séroprévalence attendue lors de la première enquête étant de 10%. Une taille de grappe de 30 individus et un coefficient de corrélation intra grappe de 0,05 ont été également considérés avec une puissance à 80%. La commande Stata ci-après a été utilisée :

« power twoproportions 0.1 0.2, m1(30) m2(30) rho(0.05) power(0.8) »

Une taille de 1 020 individus a été trouvée, soit 510 individus par strate (Est-Ouest). En estimant le taux de réponse à 80%, la taille finale de l'échantillon est arrondie à 1 240, soit 620 individus par strate.

b. Technique d'échantillonnage

Les zones de santé de la ville de Kinshasa seront réparties en deux strates en tenant compte de leur localisation géographique Est-Ouest et la prévalence de l'épidémie.

Dans chaque strate, un échantillonnage en grappes à 2 degrés (aires de santé et ménages) sera réalisé. La zone de santé (ZS) sera considérée comme le domaine d'étude et dans chaque domaine d'étude, les aires de santé (AS) vont constituer des grappes. L'unité de sondage primaire sera la grappe, en l'occurrence l'aire de santé et l'unité secondaire d'échantillonnage est le ménage. Le nombre de grappes à échantillonner est déterminé par la formule suivante :

$$\text{Nombre de grappes} = \frac{\text{taille de l'échantillon}}{\text{nombre moyen d'individus par grappe}}$$

En se basant sur cette formule, le nombre d'avenues et d'aires de santé à échantillonner seront déterminés comme suit :

Paramètres	Formule	Application	
Nombre moyen d'individus par grappe	Population de Kinshasa/ (Nombre total d'aires de santé)	(10 000 000/380)	26316
Nombre de ménages par grappes	Nombre moyen d'individus par grappe / (Nombre moyen d'individus par ménage)	26316/5	5283
Nombre de grappes à échantillonner par domaine d'étude	Taille de l'échantillon/Nombre moyen d'individus par grappe	(1240/5283)*100	23,5%
Nombre moyen de grappes par domaine d'étude	Nombre total de grappes/Nombre de domaine d'étude	380/35	10,8
Nombre d'avenues à échantillonner	Le nombre d'avenues que compte la ville de Kinshasa étant inconnu, nous allons choisir le nombre théorique de 30% d'avenues sur la liste exhaustive d'avenues par grappe.		

En appliquant la proportion de nombre de grappes à échantillonner par domaine d'étude (23,5% de 10,8), nous obtenons 3 aires de santé à tirer de manière aléatoire dans chaque zone de santé. La fonction alea () nous permettra de sélectionner le nombre de grappes voulu sans possibilité de doublons sur Microsoft Excel.

La taille moyenne d'individus par grappe étant de 30, l'échantillon sera constitué de 42 grappes. Chaque zone de santé comprenant 3 grappes, un total de 14 zones de santé seront sélectionnées pour l'enquête à raison de 7 zones de santé par strate.

En pratique, les zones de santé de la ville de Kinshasa seront scindées en deux strates en fonction de leur localisation géographique et du nombre des cas rapportés. Dans chaque strate, 7 zones de santé seront tirées de manière aléatoire.

Puis dans chaque zone de santé, un échantillonnage en grappes à 2 degrés sera effectué de la manière suivante :

- Au 1^{er} degré (unité de sondage primaire) dans chaque ZS : tirage aléatoire simple de 3 grappes représentées par les aires de santé sur base de la liste exhaustive des aires de santé
- Au 2^{ème} degré (unité de sondage secondaire) : un tirage systématique de 8 ménages sera effectué dans chaque aire de santé. Estimant que 50% de la population est âgé de plus de

18 ans, dans 50% des ménages, tous les résidents seront invités à participer à l'étude et dans les 50% autres, seuls les individus de 18 ans et plus seront invités à participer. Chaque enquête est indépendante l'une de l'autre. Le processus de sélection des aires de santé, avenues et ménages sera répété à chaque enquête.

5. Collecte des données

a) Mobilisation de la communauté

La mobilisation de la communauté pour assurer un taux de réponse élevé est une priorité pour l'organisation de l'étude. L'accord de la communauté sera donc recherché afin d'avoir son soutien pour le bon déroulement de l'enquête. Les chefs de localités ainsi que les leaders influents seront visités au moins 2 semaines avant le début de l'enquête par les équipes, afin d'augmenter l'acceptation des enquêteurs par la communauté. L'opportunité sera prise pour expliquer les objectifs de l'étude, la méthodologie de sélection des ménages, les questionnaires qui seront utilisés ainsi que les déplacements des équipes à l'intérieur des localités. Il sera également question de discuter des problèmes et des préoccupations potentielles notamment, prévenir les rumeurs. Les équipes répondront à toutes questions éventuelles concernant le déroulement de l'enquête avant de demander leur consentement pour mener les entretiens dans leurs quartiers. En outre, il sera clairement expliqué aux chefs de localités et aux leaders influents qu'ils sont libres d'autoriser ou de refuser la participation de leur localité respective dans cette enquête, sans que cette décision n'ait de conséquences sur l'accès aux soins, la qualité de la prise en charge COVID-19 ou toute autre action de contingence de l'épidémie, menée par la riposte COVID-19.

Un dialogue continu avec ces parties prenantes sera important pour maintenir l'engagement de la communauté avant et pendant l'étude.

b) Equipes de terrain

Les équipes de terrains seront au nombre de 12 (une équipe pour 7 Aires de Santé) et comprendront chacune 3 personnes dont 2 enquêteurs et 1 préleveur. De plus, il y aura 4 superviseurs, dont 2 assurant chacun la coordination de 6 équipes d'enquête par strate (Est-Ouest) et 2 autres assurant la supervision des enquêteurs pour l'un et la supervision des préleveurs pour l'autre.

Une formation sera dispensée à tous les superviseurs et enquêteurs pour les familiariser avec les différentes étapes et composantes de l'étude. Au cours de cette formation, un décriptage approfondi du questionnaire (question par question) et des formulaires de consentement éclairé sera réalisé. Les enquêteurs se familiariseront avec les objectifs de l'étude, le remplissage des outils de collecte de données (Tablettes et GPS), ainsi que l'approche à suivre à l'intérieur du ménage. Pendant la formation, les exercices de jeu de rôle seront essentiels pour pratiquer l'introduction du sondage, obtenir le consentement éclairé et écrit, poser des questions et remplir des formulaires. A l'issue de la formation, les équipes d'enquête seront déployées dans les zones de santé non sélectionnées pour l'étude afin de pré-tester les outils de collecte.

c) Collecte des données proprement dites

- **Les enquêtes sérologiques et les questionnaires**

Dans un premier temps, les enquêteurs présenteront l'étude aux membres du ménage (cf. notice d'information sur l'étude). Puis la non-opposition du chef de ménage sera recueillie directement sur le questionnaire ménage (cf. questionnaire ménage).

Les données seront collectées à l'aide de tablettes grâce à l'application Epicollect qui reprendra la version électronique du questionnaire d'enquête. Les données seront ensuite transférées vers le logiciel d'analyses statistiques. Toutes les informations seront transcrites en français et transférées vers une base de données protégées par un mot de passe.

Le chef de ménage ou tout autre membre adulte du ménage aidera l'enquêteur à remplir le questionnaire de ménage, qui reprend les questions relatives aux membres du ménage, leur mois/année de naissance, leur relation avec le chef de ménage, leur âge (voir annexe A). Le questionnaire ménage sera utilisé aussi bien pour la description de la composition du ménage que pour des analyses descriptives sur la population.

L'éligibilité sera vérifiée auprès de chaque personne avant le processus d'information et de consentement individuel. Pour tout membre du ménage qui est éligible et intéressé à participer, l'enquêteur obtiendra ensuite le consentement éclairé de l'individu pour participer à l'étude dans un espace privé. Après le processus de consentement et/ou d'assentiment, l'enquêteur mènera des entretiens individuels à l'aide de questionnaires individuels, en face à face dans une zone privée à l'intérieur ou proche du ménage. Les questions reprises dans le questionnaire sont les suivantes.

- Caractéristiques démographiques générales : sexe, âge, éducation, état civil, travail
- Caractéristiques cliniques : présence ou non de symptômes liés à l'infection à SARS-COV-2
- Autres : comportement favorisant la santé (recherche de soins), contact antérieur avec un malade confirmé ou probable, diagnostic confirmé ou probable de l'infection à SARS-COV-2.

Les deux questionnaires (des ménages et des individus) seront prétestés et validés sur 10 personnes (soit 2 à 3 ménages) par chaque équipe, avant l'implémentation de l'enquête. Les questionnaires seront administrés aux participants dans la langue qui leur convient le mieux (Français ou l'une des 4 langues nationales).

Les équipes utiliseront des récepteurs GPS pour établir et enregistrer les coordonnées géographiques de chaque centre des grappes. Toutefois, à des fins logistiques, et uniquement pour la durée de l'étude, les ménages seront géographiquement localisés et identifiés sur une carte, mais ces informations ne seront pas conservées ou enregistrées dans une base de données.

6. Encodage et analyse des données

a. Encodage

Les données quantitatives seront collectées à l'aide d'un formulaire électronique sur Epicollect5 puis transférées vers le logiciel d'analyses statistiques par un opérateur de saisie expérimenté, formé et supervisé par l'investigateur principal.

Tous les fichiers électroniques seront protégés par un mot de passe avec un accès limité. Un nettoyage des données sera effectué pour vérifier les incohérences dans la saisie des données et les réponses.

b. Méthodes statistiques

Pour chaque participant, plusieurs dimensions seront considérées. Les analyses descriptives seront pondérées pour prendre en compte la probabilité de sélection de l'échantillonnage en grappe et seront présentées avec un intervalle de confiance à 95%. Les statistiques du test t simples et du chi² de Pearson seront utilisées pour comparer les résultats descriptifs continus et catégoriels, respectivement. Les résultats seront calculés avec les intervalles de confiance correspondants à 95 % et stratifiés par groupe d'âge, sexe et zone géographique. Le coefficient de corrélation intra-famille effectif sera calculé à l'issue de la première enquête.

Les résultats sérologiques seront stratifiés par âge, zone géographique et pondérés pour prendre en compte l'effet lié à l'échantillonnage. Des modèles logistiques multivariés évalueront l'association entre la séroprévalence et facteurs de risque dans chaque étude. Après la première enquête, une analyse classique transversale sera conduite suivie d'une approche incrémentielle en poolant les données issues des enquêtes ultérieures et en introduisant une covariable codant pour la prévalence à chaque enquête. L'hétérogénéité spatiale sera prise en compte.

La dynamique de l'épidémie sera modélisée en utilisant des modèles linéaires généralisées (11) . De plus, les modèles de diffusion de l'épidémie qui seront disponibles, publiés par d'autres équipes, seront appliqués aux données afin d'estimer les paramètres de diffusion observés à Kinshasa (incidence cumulée, temps de doublement, variabilité spatiale de la transmission).

c. Critères de jugement

Le critère de jugement principal

- La différence de séroprévalence entre deux enquêtes et stratifiée par zone géographique (Est-Ouest)

Critères de jugement secondaires :

- La séroprévalence définie comme la proportion des participants par classe d'âge présentant des anticorps IgG anti-SARS-CoV-2 (Obj. 1)
- La proportion de cas symptomatiques définie comme la présence de symptômes de l'infection au virus SARS-CoV-2 depuis le début de l'année parmi les participants avec une sérologie SARS-CoV-2 positive (Obj. 2) :
 - Fièvre
 - Fatigue
 - Toux (sèche)
 - Rhinite
 - Mal de gorge
 - Difficulté à respirer
 - Douleurs thoraciques
 - Douleurs musculaires
 - Nausée/Vomissement
 - Signes dermatologiques, anosmie, dysgueusie
 - Asthénie
 - Anorexie
- La proportion de cas asymptomatiques parmi les participants avec une sérologie SARS-CoV-2 positive (Obj. 2)
- Le nombre de cas antérieurement confirmés par test PCR (Obj. 3).
- Le nombre de cas antérieurement classés probables ([https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-\(2019-ncov\)](https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov))) (Obj.3).
- La proportion d'individus qui sont allés consulter un prestataire de soins de santé pour un ou plusieurs de ces symptômes. (Obj 3.)
- Parmi les individus avec une sérologie positive, la proportion qui a été diagnostiquée infection à SARS-CoV-2 et confirmée par test PCR, la proportion qui a été diagnostiquée infection probable et la proportion qui n'a pas été diagnostiquée (Obj.3).
- Le contact avec un patient suspect ou confirmé d'infection au virus SARS-CoV-2 (Obj.4).

7. Collecte et transport des échantillons

Après l'entretien individuel, l'enquêteur proposera un prélèvement sanguin qui consiste à collecter environ 5 ml de sang veineux dans un tube vacutainer contenant de l'EDTA, sur chaque participant. Les tubes EDTA ainsi collectés seront identifiés par un numéro d'identification unique correspondant au numéro attribué sur la fiche d'investigation du participant. En plus de l'identifiant, il sera marqué sur le tube le nom, l'âge et le sexe du participant. Le prélèvement sanguin sera réalisé par un personnel qualifié et formé en matière de biosécurité lié au contexte de l'étude. Des Conseils pré- et post-test seront prodigués à tous les participants à l'étude. Les échantillons ainsi prélevés seront placés dans un triple emballage avec accumulateur de froid et transportés à la température de 4°C jusqu'à l'INRB pour analyse.

8. Analyses de laboratoire

Les tests sérologiques seront effectués à l'INRB à l'aide d'un automate utilisant la technique de Luminex. Tous les échantillons seront testés à la recherche des IgG dirigés contre les protéines Spike et NP de SARS-CoV-2, de SARS-CoV-1 et de MERS-CoV par des techniciens de laboratoire formés et qualifiés. Le contrôle de qualité interne sera assuré en utilisant des contrôles négatifs et positifs dans toutes les procédures de laboratoire.

Le reste des échantillons seront stockés à l'INRB sous la responsabilité technique du chef du laboratoire de virologie de l'INRB.

Les tests sérologiques utilisés ici sont des outils de laboratoire et non des outils de diagnostic. Ils pourront ainsi permettre de définir si les personnes ont été en contact avec le SARS-CoV-2 responsable de l'épidémie de Covid-19 mais pas de savoir si elles sont encore infectées. Aucun certificat d'immunité contre le virus du SARS-CoV-2 ne sera transmis.

Les personnes enrôlées dans l'enquête seront informées individuellement par SMS de leur résultat un mois après l'enquête et chacune d'entre-elle aura la possibilité de venir récupérer le résultat de sa sérologie en se rendant au laboratoire de l'INRB.

Les éventuels cas symptomatiques (répondant à la définition de cas) rencontrés au cours de l'étude seront référés à l'équipe de riposte contre la Covid-19 pour une prise en charge adéquate (investigation et éventuel prélèvement pour le diagnostic moléculaire du SARS-CoV2).

9. Considération éthiques

a. Consentement éclairé

L'étude sera conduite dans le respect de la Déclaration d'Helsinki (Principes éthiques pour la recherche médicale impliquant des sujets humains).

Le protocole de l'étude sera soumis au comité éthique de l'Ecole de Santé Publique de Kinshasa pour approbation. Les objectifs et la finalité de l'étude seront expliqués aux autorités sanitaires et politico-administratives des Zones de Santé ciblées pour approbation avant le début de l'étude.

Le consentement éclairé écrit sera préalablement obtenu, par un membre qualifié de l'équipe d'investigation, pour toutes les personnes prêtes à participer à l'étude. Le consentement éclairé d'un parent ou d'un tuteur légal sera demandé pour tous les enfants qui n'ont pas atteint l'âge légal du consentement (18 ans). Chaque participant(e) sera informé(e) que sa participation à l'étude est volontaire et qu'il ou elle sera libre de se retirer de l'étude à tout moment, sans avoir à se justifier, sans conséquences, et sans que cela nuise à ses responsabilités professionnelles, ou à sa prise en charge en cas d'infection au COVID-19. En outre, les participants potentiels à l'étude seront informés de l'orientation vers les services de soins et de traitement de la COVID-19 disponibles dans les centres de soins et de traitement les plus proches.

La non-opposition du ménage pour la participation à la recherche de ses membres sera obtenue avant tout auprès du chef de ménage ou de la personne agissant en tant que chef de ménage en son absence et sera notifié sur le questionnaire ménage par l'enquêteur (cf VI.2.a).

Cette étude sera réalisée selon la législation en vigueur sur le respect du secret médical, le traitement des données informatisées et la protection des personnes se prêtant à la recherche biomédicale.

b. Remboursement pour participation

Les participants ne seront pas remboursés pour leur participation et ne recevront aucune incitation financière pour participer à l'enquête.

Cependant, le représentant de chaque ménage recevra une prime de transport équivalent à 10 000 CDF (\$5) pour lui permettre de retirer le résultat sérologique à l'INRB. Dans le but de promouvoir l'hygiène des mains et l'application des gestes barrières, tels que recommandés par le Ministère de la santé, les participants qui le souhaitent auront aussi accès à des gels hydroalcooliques et des masques de protection.

c. Risques potentiels et avantages

1.1. Risques potentiels

L'enquête comporte un risque minimal pour les participants, lié au prélèvement d'une petite quantité de sang. Les risques identifiés sont les suivants :

- **Ponction veineuse** : elle peut entraîner une gêne, des ecchymoses, un gonflement et rarement une infection à son site. Tous les préleveurs (infirmier(e)s/laborantins) impliqués dans l'enquête seront préalablement formés à la technique de ponction et à la prise en charge de ses complications.
- tous les incidents AES qui surviennent pendant l'acte de prélèvement du sang seront immédiatement notifiés à l'investigateur principal de l'enquête et pris en charge selon le protocole national ; une assistance médicale sera rapidement fournie si un participant en a besoin.

1.2. Avantages

Cette enquête de séroprévalence comporte des avantages pour les participants et pour les communautés à long et à moyen terme tels que :

- Pour les participants, une meilleure compréhension des facteurs associés à l'infection au COVID-19 permettant de prendre des actions contribuant à réduire le nombre de nouvelles infections au niveau individuel et communautaire. Dans le cadre de l'enquête, les participants bénéficieront d'une séance personnalisée de sensibilisation sur le COVID-19, l'importance des gestes barrières et la conduite à tenir en cas de suspicion de cas.
- Tous les patients symptomatiques seront référés à l'équipe de la riposte pour un diagnostic par le test PCR. Si le résultat de la PCR est positif, le participant sera immédiatement pris en charge selon le protocole de prise en charge des patients COVID-19.
- Au niveau sanitaire, Une meilleure compréhension des principaux indicateurs de l'épidémie de la COVID-19 permettra d'orienter les actions de la riposte COVID-19 en RDC.
- La disponibilité de données précises et fiables sur l'étendue de l'épidémie à Kinshasa revêt une importance capitale pour une meilleure compréhension de la dynamique de l'infection et un choix éclairé des différentes interventions à mettre en place pour le contrôle et l'élimination de la maladie à coronavirus COVID-19. Aussi ces données pourront être utilisées pour le plaidoyer au niveau national et international.

d. Confidentialité

La confidentialité des informations concernant les participants sera maintenue tout au long de l'étude. Chaque sujet qui participe à l'étude recevra un numéro d'identification qui sera attribué par l'équipe d'investigation et noté sur la fiche d'investigation, le consentement éclairé et l'échantillon. Le lien entre ce numéro d'identification et les participants sera géré par l'équipe d'investigation et ne sera pas divulgué ailleurs.

L'INRB sera responsable de l'archivage dans un lieu sûr et approprié de tous les dossiers et de toute autre documentation liée à cette étude. Il devra également s'assurer que seul le personnel compétent et délégué pour ce travail a accès aux fichiers. A l'issue de l'étude, toute la documentation sous format papier ou électronique, sera conservée conformément à la législation locale pour une durée de 15 ans. Le dossier de l'enquête sera à tout moment disponible pour les audits internes et / ou les inspections des autorités réglementaires à l'issue de l'étude.

e. Prévention de l'infection par le virus de la COVID-19 parmi le personnel d'enquête

Tout personnel impliqué dans l'étude sera formé aux procédures de lutte anti-infectieuse (précautions standard contre la transmission par contact, précautions supplémentaires contre la transmission par gouttelettes, conformément aux directives nationales et celles édictées par l'OMS). Ces procédures devront comprendre les bonnes pratiques d'hygiène des mains et l'utilisation correcte des masques afin de limiter le risque d'infection du personnel lors d'un contact étroit avec des personnes atteintes de la COVID-19, mais également pour limiter le risque de propagation parmi les autres participants à l'enquête. En outre, les enquêteurs bénéficieront d'un test diagnostique virologique sur prélèvement naso-pharyngé avant chaque enquête. En cas de suspicion clinique de Covid-19 pendant l'étude, l'enquêteur sera référé à un centre de prise en charge.

f. Déviation au protocole

Le non-respect du protocole, des procédures opératoires standardisées, des bonnes pratiques cliniques ou des dispositions législatives et réglementaires en vigueur en République Démocratique du Congo par un membre du personnel de l'étude doit conduire rapidement à la mise en œuvre des mesures appropriées par le promoteur.

Aucune dérogation au protocole ne sera autorisée par le promoteur.

Les investigateurs s'engagent à respecter les dispositions du protocole, notamment en ce qui concerne les conditions de réalisation de la recherche et les modalités d'expression et de publication des résultats.

Toutefois, en cas de survenue d'une déviation au protocole, l'investigateur, ou toute personne qu'il aura désignée, se doit de la justifier, de la documenter et de transmettre les informations au promoteur dans les plus brefs délais.

L'ensemble des déviations au protocole sera présenté au Conseil Scientifique de la recherche.

Si une déviation grave ou délibérément répétée, de la part d'un investigateur ou d'une autre personne impliquée dans la recherche, est décelée à l'occasion du suivi de la recherche, du contrôle de qualité ou d'un audit, le promoteur mettra un terme à la participation de cette personne à la recherche.

IV. COMMUNICATION

a. Communication des résultats de l'étude

Un mois après chaque enquête, les résultats du test sérologique seront communiqués individuellement aux participants par SMS. Chaque participant aura également la possibilité de récupérer le résultat imprimé de sa sérologie en se rendant à l'INRB.

Aucun certificat d'immunité contre le virus du SARS-CoV-2 ne sera transmis.

Les résultats de chaque enquête feront l'objet d'un rapport transmis au ministère de la santé, au ministère de la recherche scientifique et à la coordination de lutte contre l'épidémie de Covid-19 de la République Démocratique du Congo. Un atelier de présentation des résultats globaux sera organisé à l'INRB.

b. Communication scientifique

Toute communication écrite ou orale des résultats de la recherche doit recevoir l'accord préalable des investigateurs coordonnateurs INRB et IRD de l'étude.

Un rapport final reprenant les objectifs de l'étude sera écrit et mis à la disposition de tous ceux qui souhaitent le consulter. Des recommandations claires seront formulées pour la coordination de la riposte et une cellule de suivi des recommandations sera également mise en place pour s'assurer que celles-ci sont bien intégrées dans la riposte.

Les stratégies de diffusion et de communication que nous envisageons sont doubles :

- Diffusion dans le pays : les résultats de l'étude seront communiqués aux experts locaux, aux décideurs politiques et aux prestataires impliqués dans la riposte contre l'épidémie actuelle de la COVID-19 afin de maximiser l'utilisation des données.
- Diffusion internationale : la communication et la publication des résultats de l'étude seront réalisées conjointement par les partenaires de l'étude. Tous ceux qui ont contribué de manière substantielle à cette recherche seront inclus en tant que co-auteurs dans les publications résultant de cette étude selon les directives de l'ICMJE. L'équipe recherchera également des opportunités lors de réunions / conférences régionales ou internationales existantes ou prévues pour partager les résultats de l'étude afin de favoriser une plus grande diffusion des résultats.

V. LIMITES DE L'ENQUETE

Malgré tous les efforts investis dans la préparation de cette enquête, on peut identifier certaines limites. Il peut y avoir un biais de sélection provenant de l'estimation approximative de la population générale par extrapolation, dans la province de Kinshasa¹. La taille de la population cible peut avoir été surestimée ou sous-estimée et donc modifier la taille de l'échantillon et les résultats.

VI. ACCES AUX DONNEES ET ECHANTILLONS BIOLOGIQUES

¹ En RDC, le dernier recensement scientifique a été réalisé en 1984. Depuis, la taille de la population est estimée par extrapolation.

L'ensemble du matériel collecté en application du protocole, c'est-à-dire les données de la recherche et les échantillons biologiques, est placé dès le début de la recherche sous la responsabilité de l'INRB. Il reste sous sa sauvegarde pendant toute la recherche et au-delà.

Toute utilisation du matériel biologique dans le cadre de la recherche non inscrite dans le protocole, ses annexes et amendements devra faire l'objet d'une demande au conseil scientifique.

Les échantillons biologiques seront stockés à l'INRB à la fin de l'étude.

Les données de l'étude seront conservées 2 ans en base active à partir du début de l'étude et 15 ans après la fin de l'étude en base d'archivage sous la responsabilité de l'INRB, par délégation du promoteur.

VII. ROLES ET RESPONSABILITES

Les ressources humaines prévues pour l'étude sont les suivantes	
Investigateur principal	
Enquête épidémiologique	1 superviseur activités épidémiologiques
	2 superviseurs des axes (Est et Ouest)
	36 enquêteurs épidémiologistes (3 par équipe dont un en charge des prélèvements ; une équipe pour 3 zones de santé)
	1 gestionnaire de données
Enquête biologique	1 superviseur activités biologiques
	4 laborantins

L'investigateur principal est le responsable de la version finale du protocole, de la qualité générale de l'enquête, de l'analyse des données, ainsi que du rapport final.

L'investigateur principal s'assurera que les tâches suivantes sont effectuées :

- Préparation de tous les documents nécessaires (protocole, questionnaire, formulaires de consentement éclairé, etc.) pour l'enquête
- Préparation de la composante de terrain de l'étude (formation des équipes d'enquête, logistique, matériel)
- Suivi du déroulement de l'enquête dans les ménages
- Qualité des données recueillies dans l'outil Epicollect5
- Analyse des données
- Rédaction de rapports intermédiaires et du rapport final

Les **enquêteurs** auront pour tâche de mener les investigations dans les ménages. Chaque équipe sera composée de trois enquêteurs (dont un en charge des prélèvements). Pour finaliser la partie terrain dans un délai raisonnable, nous aurons besoin de 6 équipes d'enquêteurs (1 pour 7 grappes) composées de 3 personnes chacune, idéalement avec au moins un homme et une femme. Les enquêteurs vont travailler en raison des 2 zones de santé par jour pour une durée de 7 jours. Donc, chaque équipe aura à enquêter dans une grappe par jour.

Le **gestionnaire de données** aura pour tâche de configurer les tablettes ou smartphones Android pour la collecte des données de l'enquête. Il sera aussi responsable de centraliser les données

collectées sur l'application Epicollect5 et de veiller à la complétude, la cohérence et la qualité générale de ces données. Enfin, il devra également s'assurer que les tablettes sont bien chargées et prêtes à être utilisées le lendemain.

Les **Préleveurs** auront pour rôles de prélever le sang, de veiller à sa conservation dans les conditions optimales et au transport vers l'INRB selon la SOP en vigueur.

L'avancée de l'enquête sur le terrain dépendra de la fluidité des mouvements, des contraintes de sécurité et de la disponibilité de la population d'étude. Les enquêteurs travailleront cinq jours par semaine et se reposeront le weekend end pour ne pas nuire à la qualité du travail réalisé lors des entretiens.

Critères de sélection pour les enquêteurs :

- Savoir lire, écrire et parler couramment le Français ET le Lingala et/ ou une autre langue nationale ;
- Être disponible pour toute la durée de l'enquête, incluant les jours de formation et d'enquête ;
- Être motivé à participer à l'enquête ;
- Ne pas être biaisé dans les attentes concernant les résultats de l'étude ;
- Avoir une connaissance de la COVID-19 et du contexte social et culturel de Kinshasa ;
- Être une personne sociable, flexible et rigoureuse ;
- Avoir une expérience des enquêtes similaires dans des environnements complexes serait un avantage.

VIII. OBLIGATIONS DES INVESTIGATEURS

Conformément aux bonnes pratiques cliniques visant à garantir la qualité de la recherche, chaque investigateur s'engage à :

- Respecter les droits des participantes et garantir leur sécurité et leur bien-être ;
- Assurer sa disponibilité et celle de son équipe ;
- Assurer que ses possibilités de recrutement sont compatibles avec la réalisation de l'étude ;
- Prendre la responsabilité d'organiser les structures techniques pour la mise en place des circuits spécifiques à la recherche (consultations, prélèvements) ;
- Recueillir et archiver en lieu sûr le consentement écrit des participants ;
- Assurer le respect du protocole et veiller à la qualité des données ainsi qu'à leur transmission régulière entre les équipes responsables de la méthodologie et de la gestion de l'étude dans les délais préconisés ;
- Permettre le monitoring régulier de l'étude par la personne adéquate qui devra avoir accès aux documents-source des participants ;
- Accepter l'inspection de la recherche par les Autorités de Santé qui y sont autorisées.

À tout moment, le chef de projet de l'étude ou les investigateurs coordonnateurs peuvent être contactés pour toute question relative au protocole, à son application pratique ou aux conduites à tenir devant certains événements.

XI. ECHEANCIER DE LA RECHERCHE

L'étude va durer un an à partir de l'accord du comité d'éthique.

Les trois enquêtes seront espacées entre elles de 6 semaines, la première débutant vers mi-October 2020, la 2^e vers fin-novembre 2020 et la 3^e vers mi-janvier 2021. Après formation des enquêteurs, chaque enquête représentera une collecte de données de 7 jours.

Le protocole sera soumis au comité d'éthique au courant du mois de septembre 2020, et la préparation de l'étude (recrutement, formation, pré test des questionnaires) se déroulera au cours de la période comprenant la deuxième quinzaine du mois de septembre et le début du mois d'octobre.

Le chronogramme est résumé dans la figure ci-dessous.

Activités principales	2020					2021							
	9	10	11	12	1	2	3	4	5	6	7	8	
Préparation de l'étude (recrutement, formation, pré-test)	X												
Préparation des sites, Mobilisation de la communauté	X												
Collecte des données et d'échantillon		X	X	X	X								
Nettoyage des données /Rapport intérimaires après chaque enquête		X	X	X	X	X							
Analyses de laboratoire		X	X	X	X	X	X						
Analyses de données		X	X	X	X	X	X	X					
Diffusion des résultats préliminaires			X	X	X								
Préparation, soumission et révision des articles						X	X	X	X	X	X	X	

XV REFERENCE BIBLIOGRAPHIQUES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;
2. Aylward, Bruce (WHO); Liang W (PRC). Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). WHO-China Jt Mission Coronavirus Dis 2019. 2020;
3. Day M. Covid-19: identifying and isolating asymptomatic people helped eliminate virus in Italian village. *BMJ*. 2020 Mar 23;m1165.
4. Kimball A, Hatfield KM, Arons M, James A, Taylor J, Spicer K, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. *MMWR Morb Mortal Wkly Rep*. 2020 Apr 3;69(13):377-81.
5. World Health Organization. Population-based age-stratified seroepidemiological investigation protocol for COVID-19 virus infection. *World Heal Organ*. 2020;
6. Dimeglio C, Loubes JM, Deporte B, Dubois M, Latour J, Mansuy JM, Izopet J. The SARS-CoV-2 seroprevalence is the key factor for deconfinement in France. *J Infect*. 2020 Apr 28. pii: S0163-4453(20)30242-5. doi: 10.1016/j.jinf.2020.04.031
7. Perera RA, Mok CK, Tsang OT, Lv H, Ko RL, Wu NC, Yuan M, et al. Serological assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), March 2020. *Euro Surveill*. 2020 Apr;25(16). doi: 10.2807/1560-7917
8. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. *J Travel Med*. 2020 Mar 13;27(2).
9. Zhou T, Liu Q, Yang Z, Liao J, Yang K, Bai W, et al. Preliminary prediction of the basic reproduction number of the Wuhan novel coronavirus 2019-nCoV. *J Evid Based Med*. 2020 Feb 12;13(1):3-7.
10. WHO Global Infection Prevention and Control Network. Infection prevention and control during health care when COVID-19 is suspected. 19/03/2020. 2020.
11. McCullagh P, Nelder JA. *Generalized Linear Models*, Second Edition. Taylor & Francis; 1989. (Chapman & Hall/CRC Monographs on Statistics & Applied Probability).

Annex 3

RESEARCH STUDY PROTOCOL

Sero-prevalence and sero-conversion study of health care workers and their households,

Democratic Republic of the Congo

Acronym: PRESTACOV (prestataires de soins dans le COVID-19)

V2.4 15/06/2020

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Statement of Compliance & Confidentiality

The information contained in this study protocol is privileged and confidential. As such, it may not be disclosed unless specific permission is given in writing by the ITM or when such disclosure is required by federal or other laws or regulations. These restrictions on disclosure will apply equally to all future information supplied which is privileged or confidential.

Once the final protocol has been issued and signed by the Investigator(s) and the authorized signatories, it cannot be informally altered. Protocol amendments have the same legal status and must pass through the mandatory steps of review and approval before being implemented.

By signing this document, the Investigator commits to carry out the study in compliance with the protocol, the applicable ethical guidelines like the Declaration of Helsinki, the ESF/ALLEA Code of Conduct for Research Integrity, and consistent with international scientific standards as well as all applicable regulatory requirements. The Investigator will also make every reasonable effort to complete the study within the timelines designated.

COORDINATING INVESTIGATOR

Date:

Title, Name:

Signed:

1. INTRODUCTION

1.1 Background

The novel SARS-CoV-2 (Severe Acute Respiratory Syndrome-associated Coronavirus type 2) , belongs to the betacoronavirus subfamily and is closely related to SARS-CoV-1 that caused the SARS epidemic in 2002-2003 and more distantly related to various human coronaviruses that have been circulating in the population for a long time and are associated with fairly mild respiratory symptoms. SARS-CoV-2 is rapidly spreading across the world causing a condition called Coronavirus disease 2019 (COVID-19) whose symptoms range from mild respiratory symptoms to a serious, sometimes life-threatening condition requiring in-hospital treatment. Since the start of the COVID-19 outbreak in the Democratic Republic of the Congo (DRC), most cases have been found in the city of Kinshasa. Other provinces are increasingly reporting cases, although the spread is limited to main cities of affected provinces (<https://who.maps.arcgis.com/apps/opsdashboard/index.html#/b871182a4f564f0f9f3e5125bee13f62>). Surveillance/control efforts thus far have been limited to testing of COVID-19 suspected cases at a central testing facility. Contacts of COVID-19 confirmed cases are daily followed up with subsequent testing as they become symptomatic. Since testing is targeted to symptomatic individuals, and neglecting asymptomatic persons, which can be up to 50% of the infected cases (1), an important part of the transmission dynamics remains unknown.

Health care workers (HCW) are among the groups at higher risk as they are directly or indirectly exposed to COVID-19 patients. They also form a group of specific interest as they are in close contact to vulnerable patients at high risk for severe COVID-19. In addition, their illness or absence from work significantly affects the health system's ability to respond to the COVID-19 pandemic and retain its other essential functions. HCW are trained in infection prevention and control (IPC). However, it is likely that at least some of them will get infected either at their workplace(s) or elsewhere. In particular health facilities with limited access to personal protective equipment (PPE) and regular water and

sanitation facilities, IPC could even potentially be hampered, resulting in an increased risk of infection transmission among HCW, from HCW to their patients as well as to their household members. Because of this crucial role played by HCW in the transmission chain, it is of utmost importance to assess the proportion of asymptomatic infections among them.

The household members of the HCW form another group of the population which can be at increased risk, given the increased risk of the HCW and living in close contact with each other within a household. Households of HCW are also a good basis in which to study the importance of household transmission within the entire transmission dynamics of the outbreak.

This study aims to investigate SARS-CoV-2 seroprevalence and seroconversion among HCW in DRC and their household members, including asymptomatic ones, in order to generate insights into the transmission dynamics as well as the clinical presentation of the disease.

2. STUDY OBJECTIVES

Primary objectives

- Determine seroprevalence of SARS-CoV-2 infection among active HCW of hospitals and primary health care units of Kinshasa at T0;
- Determine seroprevalence of SARS-CoV-2 infection among household members of active HCW of hospitals and primary health care units of Kinshasa at T0;
- Determine incidence of SARS-CoV-2 seroconversions among HCW of hospitals and primary health care units of Kinshasa over a 3 months' time period;
- Determine SARS-CoV-2 seroconversion among household members of active HCW of hospitals and primary health care units of Kinshasa over a three months' time period.

Secondary objectives

- Identify work-related risk factors (infrastructural, availability of equipment and behavioral) for seroconversion of HCW;

- Identify socio-demographic and behavioral risk factors for seroconversion of household members;
- Determine the proportion of asymptomatic cases among seroconversions occurred during the study period among HCW as well as among their household members;
- Assess secondary attack rate among household members of symptomatic and asymptomatic HCW.
- To compare the performance of an in-house Luminex platform serologic diagnostic test, commercialized antibody ELISA test and an antibody RDT against the gold standard serological test (neutralization) in tropical settings with intense cocirculation of malaria and other infectious diseases

3. STUDY DESIGN

Prospective cohort-study, with data collection every 4 weeks over a period of 3 months (4 surveys: T0, T1, T2, T3)

4. METHODS

4.1 Study Setting, Population and Sampling Strategy

Study site will be the capital of DRC, Kinshasa.

Population of study:

- Health care workers: The criteria for inclusion are all staff working in a health care facility. This will include personnel present in the patient's room and personnel who may not have provided direct patient care but who could have come into contact with a patient's biological fluid/respiratory secretions, or with potentially contaminated objects or environmental surfaces. The term "health care worker" includes allied health workers and auxiliary health workers such as cleaning and laundry personnel, x-ray physicians and technicians, midwives, phlebotomists, social workers, laboratory personnel, admission/reception clerks, patient porters, catering and cleaning staff, medical and nursing students.

- Household members: These are the members of a household, which consists of a person or group of persons who live together in the same house, share the same housekeeping arrangements and usually eat meals together(<https://www.jstor.org/stable/23056743?seq=1>).

Selection of HCW:

The health care workers will be randomly selected in different wards of different purposively selected hospitals and first line health centers. The health zones that were selected are: Lingwala, Gombe, Limete, Ndjili and Lemba. The following is proposed:

- a. 5 hospitals were purposively selected among the ones where most COVID-19 cases are being hospitalized: Vijana (Lingwala), St Joseph (Limete), Clinique Ngaliema (Gombe), HASC (Ndjili) and CUK (Lemba). Not all of them have ICU-care for COVID-19 patients. Health care workers from the following wards will be selected:
 - i. COVID-19 care unit
 - ii. Internal medicine ward
 - iii. Outpatient/consultation ward

- iv. Vaccination/immunization ward
 - v. Emergency ward
 - vi. Labour and delivery ward
 - vii. Pediatric ward
 - viii. Surgical ward
- b. 10 health centers: two in every selected health zone. For each zone, 1 health center will be selected in areas with highest attack rates and where COVID-19 cases were diagnosed, and 1 health center in an area where few or no cases were detected. The selection of health centers is based on purposive selection by Médecin Chef de Zone, depending on frequentation and openness of health center to participate in study. Health care workers from the following wards will be selected:
- i. Consultation/outpatient ward
 - ii. Laboratory/support service (social service/reception/other)
- c. 5 ‘equipe de riposte’ , working in each of the 5 selected health zones. 10 persons per ‘equipe de riposte’ will be randomly selected.

In each ward (as stated above), 12 HCW will be selected in hospitals and 6 HCW in Health centers – in case fewer people are working in the health center, all staff will be included in the study. The same health care workers will be followed up over the 3 month period of project.

Sample size:

- In hospitals: 12 persons/ward* 8 wards *5 hospitals = 480 participants
- In health centers: 6 persons/ward*2 wards *10 health centers = 120 participants
- Equipe de riposte: 10 persons/equipe *5 zones= 50 participants

Sampling strategy for HCW in hospitals: stratified systematic random sampling, based on the staff list (of persons that worked at least one day in the month previous to the first T0 survey) in the selected ward will be done. There will be 4 strata: (1) Medical Doctor or intern (MD during specialization period) ; (2) nurse or medical assistant or midwife or nurse student; (3) lab personnel or x-ray personnel or social workers or admission/reception clerks; (4) cleaning personnel or patient transporters

Sampling strategy for HCW in health centers: stratified systematic random sampling, based on the staff list (of persons that worked at least one day in the month previous to the first T0 survey) in the selected wards. There will be 2 strata: (1) Medical Doctor or intern or nurse or midwife; (2) laborant or social worker or cleaning personnel or reception clerks

Selection of household members

All household members of participating HCW who tested COVID-19 positive in the current or one of the previous rounds (on average 5 household-members per HCW – a maximum of 20% of HCW over the 4 surveys (5%/survey) can be expected to be positive: 130 HCW with each 5 household members= max 650 persons to be followed in the last survey). Of the 8 wards selected per hospital and 2 wards per health center and per equipe de riposte, the household members of one COVID-19 negative HCW (negative in the current round + for all the previous rounds) will be surveyed – this is 10% of the HCW. This latter would result in 65 households (with each 5 household members= 325 persons).

In total 195 households (975 household members) will be surveyed for a maximum of four times.

Sampling strategy for household members of HCW: The study team will visit the houses of the HCW (as explained above) or, if deemed important due to security reasons, the household members will be asked to come to the health facility.

Sample size estimation:

We expect to have a sero-prevalence of 5% in the health care workers - for a precision of 2% (alfa error of 0.05), we need a minimum sample of 456 participants in each survey.

For the sero-prevalence estimate, we took into account a cluster-effect of 1.4, reaching 650 as sample size; and for the household members a cluster-effect of 2.1, reaching 975.

For the seroconversion HCW and household members: to show with 80% power and 95% precision, a difference of 1% seroconversion in the household members and 4% in the HCW, we need to follow up 339 HCW and 678 household members. Taking into account a probability of 20% of loss-to-follow up, our final sample of 650 HCW and 975 household members will be sufficient.

Inclusion and Exclusion Criteria

In order to be eligible, study participants **must meet the following criteria for HCW:**

- » Willing and able to provide written informed consent to provide dried blood spot in all surveys and a blood sample in the first survey and answering questionnaire
- » Health care worker as defined above
- » Being sick or having had COVID-19 is not an exclusion criterion
- » Health care worker residing in the health zone of the hospital (or if not, willing to bring household members to hospital for participation in study)
- » Health care worker foreseen to be employed (or place of internship) for the next three months in the selected health facility

In order to be eligible, study participants **must meet the following criteria for Household members:**

- » Willing and able to provide written informed consent (by respondent or assent or caretaker) to provide dried blood spot and answering questionnaire
- » Willing not to change the residence during the study period
- » Household member of included Health care workers as explained above
- » Being sick or having had COVID-19 is not an exclusion criterion

4.2 Procedures

4.2.1 Data Collection:

Participants will have unique coded identifiers that allow construction of a pseudo-anonymized database for third parties.

The identifiers will be used for the identification of the blood sample that will be collected during each survey in health workers and household members.

The cohort of participants will be followed up at 4 time moments (with four weeks in between each): T0, Month 1, Month 2 and Month 3.

A. Blood sample collection

- For health care workers:

Blood sample will be taken: a fingerprick will be done to do a RDT malaria test, and one clotted blood tube to obtain serum (of 6 ml) taken by experienced nurses or laboratory technicians following the national standard procedures. The collected specimens will be transported to the central INRB laboratory in temperature controlled (4°C) conditions. All blood samples will be processed at INRB and tested

on the Luminex platform. For the first survey, blood samples will be additionally tested with EUROIMMUN ELISA analysis, with the Rapid Diagnostic Test QuickZen (called ZenTech in DRC) and for thick blood film (malaria microscopy). Of these samples that tested positive for COVID-19 antibodies on one of the 3 tests, a confirmation test will be done with the plaque reduction neutralization test (gold standard test) in the virology laboratory of ITM, Antwerp.

- For Household members:

Dried Blood spots: A health worker will take the interview and a fingerprick to collect minimum 3 – max 6 whole bloodspots from each participant + do a malaria RDT. The blood spots (75µl each) will be made on filter paper (903 whatman protein saver cards). One paper will be taken for each individual study participant. The unique code will be written on each filter paper, together with a number 0 to 4, representing the sequence of surveys. The filter papers will be air dried (4 hours at ambient temperature) and stored per 5 participants in separate Ziplock plastic bags together with silica desiccant gel. They will be stored in a cool-box and in the end of the day taken to the central laboratory of INRB, where they will be kept in a -20 freezer.

B. Data on risk factors

Data on risk factors and on clinical history will be taken from each study participant. Separate questionnaires will be made for health workers and for household members. For children below 16 years old and people with disabilities, a caretaker will be asked to respond on the socio-demographic characteristics and behavior of them.

In each team a health worker or Field Epidemiology student will collect the information for the questionnaire. The form will be filled directly on an electronic device (tablet), using Kobotoolbox (ODK application). The questionnaires for Health workers and for household members are different, as exposure to risk factors is different. The questionnaires of the WHO-protocols are used as basis for the survey (for HCW:<https://www.who.int/publications-detail/WHO-2019-nCoV->

[HCW_Surveillance_Protocol-2020.1;](https://www.who.int/publications-detail/WHO-2019-nCoV-HCW_Surveillance_Protocol-2020.1)

and <https://www.who.int/publications->

[detail/protocol-for-assessment-of-potential-risk-factors-for-2019-novel-coronavirus-\(2019-ncov\)-infection-among-health-care-workers-in-a-health-care-setting](https://www.who.int/publications-detail/protocol-for-assessment-of-potential-risk-factors-for-2019-novel-coronavirus-(2019-ncov)-infection-among-health-care-workers-in-a-health-care-setting); for household members: [https://www.who.int/publications-detail/household-transmission-investigation-protocol-for-2019-novel-coronavirus-\(2019-ncov\)-infection](https://www.who.int/publications-detail/household-transmission-investigation-protocol-for-2019-novel-coronavirus-(2019-ncov)-infection)).

For the HCW, specific attention goes to risk factors associated with hygiene, personal protection, but also exposure to COVID-19 patients. For household members, the risk factors evaluated are mainly in the behavioral sphere, respecting or not the confinement measures and the demographic characteristics.

4.2.2 Laboratory Procedures

Blood spot filter papers will be prepared by punching two discs of 4-mm diameter, and eluted overnight in 160 μ L of PBS-TBN (dilution 1:40, PBS-1 % BSA-0.15 % Tween, pH 7.4, Sigma-Aldrich). Just before use in the immunoassay, the eluted samples will be further diluted to 1:200 in PBS-BN.

We will use an in-house developed multiplex antibody assay for the detection of anti-SARS-CoV2 antibodies. As target for the detection of anti-CoV antibodies in our Luminex platform, we have selected the main immunodominant antigens of the CoV family members: the large spike glycoprotein (S) and the nucleocapsid (NC) protein. We therefore coupled recombinant NC- and S-proteins of all currently known human CoVs (all bought at Sino Biological including SARS-CoV-2, INTERCHIM, Montluçon, France) to SeroMAP microsphere beads. These beads are carboxylated polystyrene microparticles that have been colorcoded into 100 spectrally distinct sets or regions. Each of these regions can be quickly distinguished by an xMAP® Instrument, which allows the interrogation of up to 100 different analytes simultaneously from only a small volume of sample (<10 μ L). Biotinylated anti-human IgG, IgA and IgM will be used to determine positivity. A cut-off value will be estimated based on a panel of negative control samples also spotted on filter paper.

For this, we obtained a sensitivity of 95% and 100% for NC and S proteins of SARS-CoV-2, respectively and specificity of 98% and 100% for NC and S respectively. The test has been evaluated on 74 well documented COVID-19 patients from the hospital in Antwerp,

all sampled between 14 and 25 days after onset of symptoms. The negative controls (n=52) are leftover samples from patients attending the ITM hospital in 2017, long before the outbreak. With this approach we are confident to detect and individually recognize all CoVs currently known to circulate in the human population.

However, given that cross-reaction with malaria can be expected (common in many serological assays), we will also test all field samples on malaria using a rapid diagnostic test.

Additional tests in the subsample (HCW of first survey)

In order to validate this in-house test, participants will be tested with a second test, namely an FDA approved commercialized test EUROIMMUN detecting IgG based on an ELISA technique. This test is widely used for country wide serological studies, e.g. in Germany.

A third test will be added to be validated in this tropical setting, this third test is an IgM and IgG antibody test in RDT format, named QuickZen.

The COVID-19 positive test results among health care workers in the first survey, with a maximum of 100 samples, will be re-tested by the plaque reduction neutralization test (PRNT), the gold standard for COVID-19 serology, at ITM Antwerp. The seroneutralisation test for SARS-CoV-2 is done with an in house developed protocol. Vero cells are seeded in 96-well plates and inoculated with SARS-CoV-2. Six serial dilutions of test serum are added in 8-fold replicates and cytopathic effect is scored microscopically after 5 days. NT50 and NT90 titers are calculated.

As malaria RDT does not detect always low levels of malaria parasitemia, in the subsample of HCW in first survey, the blood will also be inspected by microscopy for malaria parasites following the national laboratory guidelines.

4.3 Data Analysis

4.3.1 Sero-prevalence and sero-conversion health care workers

Sero-prevalence will be calculated at each survey moment for the entire group, separate by type of service (hospital or health centers) and also separately for the different strata of types of health personnel. Evolution over time will be followed up.

Exposure to risk factors will be looked at for positive and negative COVID-19 HCW, as is their evolution over time concerning the presence or absence of risk exposure and their seroconversion status.

The proportion of individuals showing a symptomatic and asymptomatic history of COVID-19 infection will be derived from the questionnaire and will be linked to the serologic evidence retrieved.

The evolution of the antibody response will be followed for sero-converted persons.

The hospitals/ facilities are likely to be heterogenous in nature, related to factors not collected (notably characteristics of case mix), that cannot be adjusted for. This will be accounted for in the analyses, with a clustered-analyses design to allow for robust estimates of confidence intervals.

4.3.2. Sero-prevalence and sero-conversion household members

The serostatus of the household members will be evaluated according the serostatus of the health care worker of the household. This will be stratified according the clinical presentation of the case: was the HCW symptomatic or asymptomatic and which influence this had on the secondary attack rate in the household.

The proportion of individuals showing a symptomatic and asymptomatic history of COVID-19 infection will be derived from the questionnaire and will be linked to the serologic evidence retrieved. The household investigation of sero-converted individuals will provide additional information on the spread of the virus within the household clusters.

The evolution of the antibody response will be followed for sero-converted persons.

The households are likely to be heterogenous in nature, related to factors not collected, that cannot be adjusted for. This will be accounted for in the analyses, with a clustered-analyses design to allow for robust estimates of confidence intervals.

5. ETHICAL ISSUES

5.1 Ethical (and regulatory) Review

This study will be submitted for formal review and approval to the Institutional Review Board of the ITM, the EC of the University Hospital in Antwerp and the IRB of UNIKIN. No participants will be enrolled or participant related activities performed before written approval from these bodies is obtained. The study will be carried out according to the principles stated in the Declaration of Helsinki, all applicable regulations and according to established international scientific standards.

5.2 Obtaining Informed Consent

The informed consent procedure will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, etc. If a participant (or parent or guardian) is unable to read or write, a signature from a witness to the informed consent discussion will be obtained. Study participants (or parents or guardians) will be informed that participation in the study is completely voluntary and that the participant can withdraw from the study at any time without any negative consequences. The Informed Consent forms can be found in the annexes of this protocol.

5.3 Study Safety

No investigational medicinal product will be administered or evaluated in this study, nor will it include invasive diagnostic procedures apart from the dried blood spot (fingerprick) sampling for the purpose of the sero-survey. For this sampling, following a fingerprick, a capillary blood sample will be applied on filter paper, to generate a dried blood spot. The blood volumes required for the study are less than 2% of the total blood volume of a healthy adult person and as such too small to affect the participants health. The participant might experience some discomfort from the fingerprick: he/she may develop a bruise or swelling (and rarely, an infection) at the fingerprick spot.

In the surveys in the health care workers, a bloodsample will be taken following routine procedures. The bloodvolume is small and does not affect the participants health. The participant may however experience some discomfort from the bloodsampling, as described above for the fingerprick.

In this study, we test participants for malaria infection, if this malaria test would be positive, we will refer the participant to the health facility and give him/her 5 US\$, which is the price for malaria treatment in Kinshasa. We test the patients also on COVID-19, but not on acute disease. It has been seen that antibodies only appear in the second week after infection, hence no curative actions will need to be taken ([https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-](https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19#:~:text=There%20is%20another%2C%20more,after%20onset%20of%20symptoms.)

[19#:~:text=There%20is%20another%2C%20more,after%20onset%20of%20symptoms.](https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19#:~:text=There%20is%20another%2C%20more,after%20onset%20of%20symptoms.))

In case there is a participant with acute disease during the survey, the study-team will refer them to the COVID dedicated hospitals and with permission of the participant, will inform the 'équipe de riposte'. Anyhow, we will communicate the final result of the COVID-19 serology to the participant in a confidential way respecting participants' privacy and well explaining that a positive test does not mean an acute disease. It will be aimed for to give the results to each participant in the subsequent round.

5.4 Insurance

This study is covered by the (no-fault) study insurance of ITM to cover any injury, damage or loss to study participants and which is caused directly or indirectly by their participation in the study.

6. MONITORING AND QUALITY CONTROL

This study will be implemented in close collaboration between the INRB and ITM. The partner institute INRB is responsible for the implementation of the study on their territory under the supervision of ITM.

Questionnaires will be pre-tested before start of study. Data collectors and the data manager will be trained on informed consent procedures, data collection and entry.

From the electronic data, a monthly data collection report will be generated (using a Rmarkdown script), which allows verifying the completeness and accuracy of the data (the number of non-responders, missing data, compare demographics of sample HCW with those of the source population). These reports will be used by the study monitor to strengthen the quality of data collection and entry. If the data quality of specific variables or observations cannot be guaranteed, the epidemiologist with the support of a statistician could jointly decide to exclude specific observations and/or variables, with a justification for each exclusion (to be kept in the investigator’s file and to be described and justified in the study report).

7. TIMELINES

	T0 (start 29 june)				T1 (start 27 july)				T2 (start 24 august)				T3 (start 21 september)			
	wk 27	wk 28	wk 29	wk30	wk 31	wk 32	wk 33	wk 34	wk 35	wk 36	wk 37	wk 38	wk 39	wk 40	wk 41	wk 42
Health care worker survey	X				X				X				X			
Household member survey			X	X			X	X			X	X			X	X
Labo-analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

8. DATA MANAGEMENT AND ARCHIVING

8.1 Data Management

The confidentiality of data will be ensured during data management and analysis.

For the questionnaires: The names will be written down on the questionnaire, because needed for retro-information about the results of diagnostic tests. Once the questionnaire data will be entered in an electronic database, data will be pseudonymized. No names will be used, the file containing names and codes will be kept confidential and will be in the hands of the Principal investigator and co-PI ITM. Only the PI and ITM co-PI will be authorized to consult this database in case of need for clarification. In the electronic databases, the names of cases/controls will never be disclosed. All laboratory specimens and study data will be identified by a code number.

After the laboratory analysis, results will be communicated to the participants. We will explain to the participants that a positive serological test means that he/she suffered from a past infection with SARS-CoV-2 virus and want to further explore to what extent it was transmitted to his/her household and that having been through a past infection does not imply a danger his/her health nor of the household.

For all study data: Participants will not be identified in any written or oral report, especially in relation to the data they provided. Participant's information will not be released to anybody outside the research team. No individual health facility data will be written in reports in order to avoid stigmatizing, the data will be shown per health facility type, not per individual structure.

8.2 Archiving

The Principal Investigator is responsible for ensuring a secure and appropriate location for storage of the Investigator's File and any other study related documentation present at site, as well as for ensuring that only site staff that is competent and delegated to work for the study has got access to the files.

After study completion, all the relevant study documentation should be retained in accordance with the local legislation and should be retained for a minimum period of 20 years after completion of the study. The ITM should be informed prior to destruction of the files.

Both databases will be stored on the INRB and ITM server.

8.3 Open access to research data

COVID-19 related information (sero-prevalence, individual status of infection, etc.) will not be made available openly to avoid stigmatisation, they will be made available upon request using a data sharing agreement to assure confidentiality for the individuals.

9. DISSEMINATION OF RESULTS

The results of this study will be shared with the COVID-19 task force that the Ministry of Health has set-up to monitor and control the disease in DRC.

Aggregated data will be presented during scientific meetings and published together with all researchers involved in peer-reviewed journals.

10. ANNEXES

- 1. Questionnaires (HCW; household members)*
- 2. Informed Consent forms (HCW; household members)*