Epidemiology as a tool to improve prevention of human rabies : local and global health implications of postexposure prophylaxis data, Institut Pasteur du Cambodge, 2003-2014

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EPIDEMIOLOGY AS A TOOL TO IMPROVE PREVENTION OF HUMAN RABIES

LOCAL AND GLOBAL HEALTH IMPLICATIONS OF POSTEXPOSURE PROPHYLAXIS DATA, INSTITUT PASTEUR DU CAMBODGE, 2003-2014

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Thèse de Doctorat d'Epidémiologie Clinique

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Dedication

To TVB, a Cambodian child whose untimely death by rabies at age 9 despite post-exposure prophylaxis was the triggering event for this research and the ultimate modification of WHO recommendations on rabies PEP worldwide.

To Emile Roux, medical doctor and researcher with a medical thesis on rabies prevention, whose dedication, work and ethics lay the groundwork for rabies prevention in humans.

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List of abbreviations

ASEAN	Association of Southeast Asian Nations
BBB	Blood-brain barrier
CCEEV	Cell-culture and embryonated egg-based vaccine
CNS	Central nervous system
CSF	Cerebrospinal fluid
DFAT	Direct fluorescent antibody testing
ERIG	Equine rabies immunoglobulin
FAO	Food and Agriculture Organization
GAVI	The Vaccine Alliance
GCP	Good clinical practice
HDCV	Human diploid cell vaccine
HRIG	Human rabies immunoglobulin
ICTV	International Committee on Taxonomy of Viruses
ID	Intradermal
IM	Intramuscular
IPC	Institut Pasteur du Cambodge
IPIN	Institut Pasteur International Network
IU	International units
MNT	Mean neutralizing antibody titres
NTV	Nerve tissue vaccine
OIE	World Animal Health Organization
PCECV	Purified chick embryo cell vaccine
PEP	Post-exposure prophylaxis
PrEP	Pre-exposure prophylaxis
PVRV	Purified Vero cell rabies vaccine
RABV	Rabies virus
RCT	Randomized controlled trial
RESIST	Rabies Elimination Support through Integrative Science and salvage Therapy
RFITT	Rapid Fluorescent Foci Inhibition Test
RI	Rabies index
RIG	Rabies immunoglobulin
RNA	Ribonucleic acid
RNA	Ribonucleic acid
TRC	Thai Red Cross
WHO	World Health Organization

Résumé

La rage entraîne plus de 60,000 décès par an dans le Monde, dont 800 au Cambodge, pays fortement endémique pour la rage canine.

La mort survient dans près de 100% des cas de rage, maladie évitable dans presque 100% des cas par l'accès à une prophylaxie post-exposition (PPE) antirabique adéquate et en temps utile. L'amélioration de l'accès à une PPE dans les zones rurales des pays endémiques permettra d'épargner des vies humaines à court terme.

Cette thèse en épidémiologie a tiré parti des données collectées auprès des patients consultant au centre antirabique et les chiens testés à l'Institut Pasteur du Cambodge (IPC), Phnom Penh. Suite à un bilan épidémiologique de la situation et des obstacles auxquels sont confrontés les patients cherchant à accéder à la PPE adéquate et en temps utile, cette thèse vise à contribuer à améliorer 1/ l'accès géographique et 2/ l'accès financier à une PPE pour les populations rurales du Cambodge.

Nous avons développé une stratégie originale d'identification des poches de populations à haut risque d'incomplétude vaccinale après une exposition potentielle à la rage. Ceci devrait permettre d'améliorer l'accès géographique à la PPE et se concrétiser par l'ouverture en Juillet 2018 d'un centre périphérique de prévention de la rage dans l'Ouest du Cambodge. Cette stratégie d'identification de difficultés d'accès aux soins est applicable à d'autres thématiques de santé, sous certaines conditions.

Notre rappel des patients et l'analyse des décès par rage parmi les patients n'ayant pas complété de leur propre chef le protocole PPE de 4 sessions intradermales sur 1 mois ne permettent pas de mettre en évidence une différence de mortalité par rage parmi les patients n'ayant reçu que 3 sessions sur 1 semaine, par rapport à au moins 4 sessions/1mois. Le raccourcissement du protocole à 1 semaine permet de réduire les coûts directs et indirects et l'absence de revenus pendant la durée du traitement en capitale. La mise en place de ce protocole doit s'accompagner d'un suivi d'au moins 6 mois des patients après leur prise en charge initiale.

L'ensemble de ces travaux a des implications qui dépassent le cadre du Cambodge: Dans ses recommandations d'Avril 2018, l'OMS recommande désormais ce nouveau protocole IPC– le premier protocole PPE antirabique abrégé à 1 semaine.

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Synthèse en Français

C'est à peine si chaque année, en France, un peu plus d'une centaine de personnes succombent à la rage. Mais, le spectacle émouvant d'un accès de rage, la certitude de la mort, les angoisses de la longue période d'incubation expliquent assez l'effroi qu'elle inspire. L'obscurité de sa cause, le temps si variable de l'incubation, l'absence de lésions anatomiques qui puissent rendre compte des symptômes que présente l'homme ou l'animal enragé ont, pour ainsi dire, irrité la curiosité des médecins et des vétérinaires, et c'est par centaines que l'on compte les mémoires écrits sur ce sujet. Quand on parcourt cette volumineuse littérature, on est surpris de la fréquence des contradictions et on reste étonné de voir combien peu de points importants restent définitivement acquis après tant d'efforts.

Emile Roux: Des nouvelles acquisitions sur la rage.

Thèse de Médecine. Paris; 1883

1. Partie 1: Contexte et rationnel

1.1 Pasteur et son époque

La rage canine est connue et redoutée des humains depuis la plus haute antiquité. Au cours des siècles de nombreux auteurs– cliniciens, vétérinaires, chirurgiens, pharmaciens – se sont penchés sur cette terrifiante maladie[1].

Le 6 juillet 1885, le jeune Joseph Meister devenait le premier humain à bénéficier de 13 doses successives d'une prophylaxie post-exposition (PPE) par vaccin atténué contre la rage développé par Emile Roux et Louis Pasteur[2]. Ce succès fut répété fin octobre 1885 chez Jean–Baptiste Jupille[2,3]. Le premier centre de PPE contre la rage en Asie, en Afrique ou en Amérique latine fut créé à Saigon en 1891 par Albert Calmette[4,5]. Les vaccins antirabiques furent ensuite progressivement améliorés, notamment par David Semple de l'Institut Pasteur de Kasauli en Inde en 1911[6–8]. Dans les années 1960, de nouveaux vaccins ont pu être développés sur des cerveaux de souris ou des embryons de canard ou de poulet, dépourvus de myéline[9]. Le vaccin Fermi puis Fuenzalida, développé sur cerveau de souriceau, est resté le plus utilisé à travers le monde jusqu'à la fin des années 1990 mais restait associé à des effets indésirables graves[10]. Des vaccins basés sur des cultures cellulaires ont ensuite été développés à partir des années 1970 – 1980[11,12]. Ces vaccins modernes, hautement antigéniques et extrêmement bien tolérés ont permis de conférer une meilleure protection, démontrée par des études sérologiques et cliniques, de raccourcir la durée des protocoles vaccinaux et de réduire la quantité de vaccins injectée en ayant recours à la voie intradermale plutôt qu'intramusculaire[11,13,14].

1.2 Aspects virologiques

La taxonomie (site web ICTV, 14/05/2018) attribue à l'ordre *Mononegavirales*, famille *Rhabdoviridae*, Genre *Lyssavirus*, un total de 16 espèces différentes de Lyssavirus identifiées à ce jour et 2 autres en cours de caractérisation. L'espèce la plus importante en santé publique correspond au virus de la rage classique ou *Rabies lyssavirus* (RABV), responsable de la quasi-totalité des décès humains et qui en constitue le virus prototype[15]. Tous les animaux à sang chaud sont susceptibles d'être infectés et de mourir de la rage. Cependant, certaines espèces de chauves-souris montrent une relation hôte pathogène différente de celle observée chez les mammifères non-volants et meurent moins de la rage[16]. Des analyses phylogénétiques entreprises sur l'ensemble des lyssavirus démontrent que les chiroptères constituent le réservoir animal ancestral des Lyssavirus[17]. En Afrique et en Asie, qui - notamment en Inde et en Chine - fait face au plus lourd fardeau de rage humaine dans le monde, l'immense majorité des cas de rage humaine font cependant suite à la transmission de RABV par une morsure de chien[18]. Une récente analyse met en évidence la diffusion au cours des XVIe et XIXe siècles d'un ancêtre commun aux virus de la rage canine qui circulent actuellement[19].

RABV est un virus en forme de balle, possédant un génome non segmenté constitué d'un brin unique d'ARN à polarité négative de 12 000 nucléotides de longueur[20]. Ce génome comprend 5 gènes qui codent pour cinq protéines: Nucléoprotéine (N), Phosphoprotéine (P), protéine de Matrice (M), glycoprotéine (G) et polymérase (L)[21] . La glycoprotéine G est responsable de l'attachement du virus aux cellules musculaires ou neuronales[22]. Après l'adhésion du virus à la membrane cellulaire et l'endocytose, le complexe ribonucléoprotéine est libéré[23,24]. S'ensuit la transcription dans la cellule des gènes et la traduction des cinq protéines ainsi que la réplication de l'ARN. Les protéines et l'ARN sont finalement réassemblées en nouveaux virions RABV qui bourgeonnent de la cellule infectée avant d'être séparés par l'action de la protéine M[25].

1.3 Physiopathologie et anatomopathologie

Dans la majorité des cas, le virus se multiplie dans les cellules musculaires[26] et le derme[27] au niveau du site d'inoculation. L'inoculation de RABV va déclencher au niveau des tissus l'activation de mécanismes inhibiteurs tels que les microARN et des réponses immunitaires innées[28,29]. En cas d'échec des mécanismes de contrôles locaux et d'un inoculum suffisant, le RABV peut se développer puis franchir la plaque neuromusculaire, dépourvue de gaine de myéline, avant de progresser à l'abri dans le nerf correspondant, sensitif ou moteur[30,31]. La diffusion hématogène n'a pas été démontrée[32]. Le RABV remonte le long de l'axone pour se

répliquer intensément dans le neurone au niveau des ganglions rachidiens (mouvement centripète) puis progresser vers le système nerveux central (SNC)[33,34]. Dans le cas d'une morsure à la face, l'accès du virus au SNC est plus direct et rapide[31]. Les techniques actuellement disponibles ne permettent pas de mettre en évidence de lésion structurelle du cerveau chez le patient enragé. Plutôt que de la destruction des neurones, il s'agit d'un profond dysfonctionnement neuronal[35]. Ceci est suivi ou associé à une dissémination (centrifuge) via les axes nerveux, notamment vers les glandes salivaires[29,36]. Enfin, Le virus RABV peut-être inoculé directement à proximité d'un nerf au niveau d'une jonction neuromusculaire, ce qui est alors apparemment associé à une période d'incubation courte[37].

1.4 Aspects cliniques de la rage

La rage et transmise de manière inconstante, même après morsure par un chien enragé. Les quelques données épidémiologiques fiables à notre disposition suggèrent que le risque global de transmission à partir d'un chien confirmé enragé en l'absence de PPE est de 279/658 (40,4%) (A. Tarantola, manuscrit en préparation). Le risque de transmission après morsure par un chien enragé va être déterminé par l'inoculum viral (présence du virus dans la salive du chien à un moment *t*, nombre et profondeur des morsures, détersion et antisepsie de la plaie), par la densité des fibres nerveuses permettant l'infection (variable selon le site anatomique des morsures) et par le temps de contact avec les tissus (et peut-être aussi en fonction du variant)[2,38,39]. Ce risque dépend donc grandement du site d'inoculation et de son innervation[38,40]. Le temps nécessaire à la réplication puis la migration du virus vers le SNC avant l'apparition des signes correspond à la période d'incubation. Cette durée est relativement longue par rapport à d'autres maladies infectieuses, permettant la mise en œuvre d'une PPE. Si des périodes d'incubation extrêmes de quelques jours ou plusieurs années ont été décrites, la majorité des cas de rage surviennent dans les quatre à huit semaines qui suivent la morsure[26,41]. La période d'incubation est plus courte en cas d'inoculum massif, de morsure à la face ou à la tête ou en cas d'inoculation directe dans les filets nerveux [42,43].

Dans la semaine précédant le début des signes, 30–70% des patients ressentent les douleurs, des paresthésies ou un prurit au niveau du site de la morsure[44]. Après cette phase prodromique, deux grandes formes cliniques inaugurales peuvent apparaître[39,45]:

1. La rage encéphalitique (« rage furieuse ») par lésion du tronc cérébral, de l'Hippocampe, du lobe temporal, dans environ 80% des cas décrits[43,46];
2. La rage paralytique (« rage muette » ou « rage tranquille ») par atteinte du système parasympathique ou des noyaux gris centraux dans environ 20% des cas[46,47].

L'hydrophobie est presque pathognomonique et classiquement synonyme de la rage[48]. Comme la fièvre, elle est fréquente mais inconstante[47,49,50]. Elle constitue cependant un élément majeur de l'autopsie verbale dans la rage[51,52]. A terme, les patients présentent des signes de l'une ou l'autre forme avec phase alternantes d'agitation et de calme, évoluant vers la paralysie des muscles respiratoires et cardiaques, mais en pleine lucidité jusqu'à la mort.

Ces données épidémiologiques et leur fréquence respective documentée sont néanmoins certainement influencées par les critères cliniques retenus dans les définitions de cas. En effet, la phase d'évolution des formes paralytiques est plus longue que dans la forme classique et la présentation clinique est proche de celle d'un syndrome de Landry-Guillain-Barré. De plus, nombre de cas de rage paralytique pourraient passer inaperçus, notamment dans les pays tropicaux où co-circulent plusieurs pathogènes responsables d'encéphalite[53–55].

La PPE et sans effet après le début des signes et il n'existe pas de traitement spécifique de la rage à ce stade. Lorsque la rage survient, l'issue est fatale dans 100% des cas (notamment dans les pays en développement de l'Ancien Monde), au bout de 2–7 jours après le début des signes. On ne connaît que quelques exceptions, avec environ 15 cas ayant survécu, dont une minorité après rage transmise par le chien et la plupart avec des séquelles graves[56]. Une réanimation agressive ne permettant que de prolonger la vie du malade atteint de rage canine de quelques jours, la prise en charge palliative s'impose généralement et doit être développée[57,58].

1.5 Diagnostic de la rage

De nombreux tests diagnostiques sont disponibles et leur choix doit tenir compte de leurs performances et surtout de l'objectif de santé publique poursuivi[59,60]. Le diagnostic de confirmation s'effectue essentiellement: (1) par immunofluorescence (DFAT) sur tissu cérébral de chien mordeur ou parfois chez le patient décédé, très performante, ou bien (2) par RT– PCR pour un diagnostic *intravitam* chez les patients suspects de rage. Cette dernière technique atteint une sensibilité de 100% lorsqu'elle est appliquée sur plusieurs prélèvements successifs, salivaires ou de biopsie nucale, en veillant à prélever des follicules pileux[47,61].

Les techniques de diagnostic ne sont actuellement pratiquées que par des laboratoires compétents souvent d'accès difficile en milieu rural de pays en développement, où la rage fait l'essentiel de ses victimes. En conséquence, ces dernières décèdent souvent à leur domicile et les cas ne sont pas rapportés au niveau central. Chez l'homme, l'autopsie verbale s'appuyant sur des définition de cas pour la surveillance[62] est considérée très performante dans la rage et peut s'avérer un outil épidémiologique très utile pour compléter les données recueillies par les réseaux plus classiques de surveillance se basant sur le diagnostic virologique[51,52,63–65]. De même, le

développement d'un algorithme prédictif syndromique de la rage chez le chien mordeur présente beaucoup d'intérêt (Mollo B et coll. manuscrit en préparation)[66].

1.6 Aspects épidémiologiques des morsures et de la rage transmise par le chien au Cambodge

Environ 80% de la population cambodgienne, estimée à 16 millions d'habitants, vit en milieu rural. Le Cambodge présente le ratio chiens: humains le plus élevé publié, évalué à 1:3 en milieu rural[67–70]. La vaccination des chiens (ou des chats) contre la rage n'est pas obligatoire au Cambodge et l'incidence de morsure par chien y est la plus élevée décrite au monde[67]. Or, la majorité des Cambodgiens par méconnaissance ou difficultés d'accès – géographique et/ou financier - n'ont pas recours à une PPE après morsure[67]. La PPE n'est en effet accessible pour la majorité de la population qu'à l'institut Pasteur du Cambodge, à Phnom-Penh[71]. C'est donc très logiquement que l'incidence annuelle publiée de la rage au Cambodge est la plus élevée qui ait été publiée au monde: elle a été estimée à 800 morts en 2007 pour une incidence de 5,8 / 100 000 (soit 3 900 morts annuellement en l'appliquant à la population Française actuelle)[70]. Depuis 2007, les populations canine et humaine ont augmenté et l'accès à la PPE ne s'est pas significativement amélioré.

1.7 La prévention de la rage chez l'humain

Les pays développés ont contrôlé la rage par la vaccination animale et la gestion des populations animales et la limitation de l'errance des chiens constitue une méthode efficace, constituant peut-être le seul exemple du paradigme « One-Health » qui ait été couronné de succès à ce jour[72–75]. La prévention de la rage chez l'homme passe aussi par la vaccination qui peut avoir lieu avant une exposition - ainsi qu'elle est pratiquée chez les professionnels de la santé animale et recommandée chez les voyageurs en zone endémique[76] (trois sessions vaccinales à J_0, J_7 et J_{20} ou J_{28} en 2017) - et par l'information des populations et des voyageurs sur les risques des morsures et sur les méthodes basées sur l'éthologie permettant d'éviter les morsures par des animaux[77].

En cas d'exposition, l'inoculum (et donc le risque de transmission) est fortement réduit par un lavage de la plaie et une antisepsie adéquats. Un protocole de PPE vaccinale selon les recommandations de l'Organisation Mondiale de la Santé (OMS) est indiqué dans les morsures superficielles ou profondes[11]. Si la morsure est profonde (Catégorie III de la classification de l'OMS) les immunoglobulines antirabiques sont indiquées dans les recommandations, mais ces immunoglobulines d'origine équine ou humaine sont très coûteuses et produites en quantité très insuffisante[71]. La suture des plaies est contre-indiquée car elle augmente le risque de transmission

et une prise en charge chirurgicale secondaire est alors préférable[11,61]. Lorsqu'elle est nécessaire, la suture ne doit être réalisée qu'après PPE et injection d'immunoglobulines dans la plaie.

Les protocoles de PPE utilisant des vaccins antirabiques modernes ont été fortement réduits en durée et en nombre de sessions par rapport aux premiers protocoles d'injections quotidiennes développés par Pasteur puis Semple ou Fuenzalida, ce dernier pouvant s'étendre jusqu'à 29 jours. Jusqu'en 2017, les protocoles de PPE validés par l'OMS (notamment les protocoles « Essen »[78] et « Zagreb »[79–83]) consistaient en plusieurs sessions d'injections intramusculaires de 0,5 ou 1 mL de vaccin sur quatre semaines, un protocole « Essen accéléré » consistant en quatre sessions sur deux semaines[84]. Le protocole le plus utilisé à l'institut Pasteur du Cambodge jusqu'en 2017 était le protocole développé par la Croix-Rouge thaïlandaise[11,85]. Ce protocole extrêmement efficace, extrêmement bien toléré et peu coûteux consistait en quatre sessions de deux injections intradermales de 0,1 mL de vaccin produit sur cellules Vero à J_0, J_3, J_7 et J_{28}. Quel que soit le protocole, le vaccin ou la voie d'administration utilisés, la PPE (administrée en temps utile selon un protocole recommandé par l'OMS) est considérée dans la littérature comme étant presque 100% efficace, même après morsure par un chien confirmé enragé (A. Tarantola, manuscrit en préparation). Tous ces protocoles basés sur des vaccins cellulaires modernes permettent d'obtenir une montée des anticorps neutralisant antirabiques à des titres considérés protecteurs ($\geq$ 0,5 UI/mL) à J_{15} chez pratiquement 100% des personnes vaccinées[86–94]. Les « échecs » relatés correspondent pour la plupart à des circonstances aggravantes (survenant en cas d'inoculum massif et de morsures profondes et multiples au niveau de la face, de la tête et du cuir chevelu avec parfois percement de la boite crânienne) et/ou au non-respect des recommandations (infiltration incomplète des plaies par immunoglobulines, suture immédiate, détersion et antisepsie inadéquates ou non réalisées, délais longs de mise en place de la PPE, rupture de la chaîne du froid)[61,95].

1.8 Améliorer l'accès à une prophylaxie post-exposition dans les pays en développement

La rage est responsable d'au moins 60 000 morts évitables chaque année à travers le Monde, soit cinq fois le nombre de morts dus à l'épidémie d'Ebola en 2014-2016 en Afrique de l'Ouest[96]. Lorsqu'un adulte assurant les revenus du ménage meurt de la rage, la famille entière peut basculer dans la pauvreté, parfois pour plusieurs générations.

On estime à 30 M le nombre de personnes qui reçoivent une PPE à travers le Monde chaque année[18,97]. On ignore cependant combien de personnes justifieraient d'une PPE chaque année à travers le monde mais n'y ont pas accès.

A terme, le contrôle de la rage humaine activement soutenu par l'Organisation Mondiale de la Santé[98,99] et l'ASEAN[100] pourrait être obtenu par la vaccination de tous les chiens, à l'instar de ce qui a été accompli dans les pays désormais développés, y compris en Asie[101,102], mais aussi dans des pays en développement en Asie[103] et à travers l'Amérique du Sud[104]. D'ici là et à plus court terme, le fardeau de mortalité due à la rage ne pourra être très fortement réduit que par un meilleur accès à une PPE adéquate et en temps utile dans les zones endémiques des pays en développement.

Un accès élargi et facilité de la population à une PPE adéquate et en temps utile constitue donc au Cambodge un enjeu majeur de santé publique, à la fois en termes de mortalité et de dépenses de santé pour des familles rurales et pauvres qui sont les plus durement touchées. Ce meilleur accès passe par une amélioration de l'accès géographique et l'identification de zones mal ou non desservies, dans lesquelles des centres périphériques antirabiques peuvent être établis. Il passe également par un raccourcissement du protocole de PPE, ce qui - en diminuant les doses, la durée nécessaire et les trajets - permettrait par la réduction des coûts directs et indirects de le rendre significativement plus accessible financièrement aux populations rurales et/ou démunies au Cambodge et à travers le Monde.

Après un bilan de la situation et des difficultés d'accès à la PPE adéquate et en temps utile au Cambodge, ces deux points constituent les deux leviers potentiels d'action de cette thèse soutenue à l'Université Paris Diderot (Paris 7) - Université Sorbonne Paris Cité portant sur «L'EPIDEMIOLOGIE COMME OUTIL POUR L'AMELIORATION DE LA PREVENTION DE LA RAGE HUMAINE: IMPLICATIONS LOCALES ET MONDIALES DES DONNEES DE PROPHYLAXIE POST-EXPOSITION, INSTITUT PASTEUR DU CAMBODGE, 2003 – 2014 ».

2. Partie 2: Obstacles à l'utilisation du vaccin et des immunoglobulines antirabiques au Cambodge

Un bilan des difficultés d'accès existantes à la prophylaxie antirabique post-exposition au Cambodge

2.1 Introduction

Le Centre de Prévention de la Rage de l'Institut Pasteur du Cambodge (cpr@ipc) a été institué en même temps que l'IPC a rouvert, le 27 Mars 1995. Il est mentionné spécifiquement dans l'accord signé entre le Réseau International des Instituts Pasteur et les autorités de santé cambodgiennes. La première année pour laquelle des données complètes sont disponibles est

l'année 1996, faisant état de 8 607 patients consultant pour prophylaxie post-exposition (PPE), parmi lesquels 76% étaient des résidents de Phnom-Penh.

En 2013, nous avons cherché à réactualiser les données concernant la situation et les services rendus à la population, pour identifier des marges de progression, si applicable.

2.2 Méthode

Cette étude a été menée en deux temps. Nous avons d'abord cherché à réactualiser nos données sur les consultations pour PPE au cpr@ipc. Ceci a été fait par une extraction et une analyse des données PPE pour l'année complète écoulée (2012). Nous avons ensuite tenté de documenter le nombre de consultations pour PPE à travers le Cambodge grâce à une enquête par Internet.

Les données ont été extraites de la base globale de l'IPC. Cette base de données sous EpiData (EpiData Association, Odense, Danemark) sert d'outil d'évaluation initiale de risque et de suivi clinique. Elle est renseignée lors de la prise en charge clinique quotidienne par la même équipe de soignants formés qui remplissent un questionnaire standard. Les caractéristiques sociodémographiques des patients et la nature de leurs plaies (nombre et site anatomique…) sont renseignés, ainsi que le statut du chien (aspect malade, morsure non-provoquée…) et la nature et délais de la PPE reçue. Ces données ont été importées et analysées à l'aide de Stata 13 (Stata Corp., College Station, TX, USA). Nous avons également examiné les bons de commande IPC pour les doses de vaccin et d'immunoglobulines antirabiques pour 2012.

Nous avons contacté le laboratoire pharmaceutique qui vend et distribue au Cambodge le vaccin antirabique produit à base de cellules Vero. Ce laboratoire avait des données sur le nombre de doses qu'il vendait chaque année dans le pays, ainsi qu'une estimation du nombre total de doses de vaccin antirabique utilisées chaque année au Cambodge. De plus, il possédait une liste des institutions dans le pays qui commandaient et administraient chaque année des doses de vaccin et d'immunoglobulines antirabiques au Cambodge. Ces institutions ont été contactées par l'auteur à l'aide de SurveyMonkey, (SurveyMonkey, Palo Alto, CA, USA) afin de documenter quantitativement le nombre de PPE administrées. Ces données ont ensuite été agrégées et anonymisées. Des données sur le coût d'achat de vaccin et d'immunoglobuline ont été collectées via Internet.

2.3 Résultats

Au total, 20 610 personnes ont consulté à l'IPC pour PPE après avoir été exposées à un animal potentiellement enragé en 2012. Ces patients étaient originaires de 22 des 23 provinces que

comptait le Cambodge à l'époque. En 2012, 98,4% des patients étaient originaires de seulement 10 de ses provinces (y compris 41% de Phnom Penh) alors que ces 10 provinces représentent 66,8% de la population totale du Cambodge. Parmi les 20 609 patients documentés pour le type d'exposition, six (0,03%) ont consulté pour griffure, 20 (0,1%) après qu'un animal ait léché leur peau lésée, un après avoir été griffé et mordu et 20 582 (99,9%) après avoir été mordu par un animal potentiellement enragé. Parmi ces morsures, 6 362 (30,9%) ont été considérées profondes (Catégorie III de la classification de l'OMS[11]) et 14 220 ont été considérées superficielles (Catégorie II[11]). Neuf pour cent des patients de Catégorie III ont reçu des immunoglobulines antirabiques d'origine équine (ERIG).

L'enquête sur le réseau de distribution des vaccins a identifié cinq centres antirabiques autres que l'IPC et a reçu une réponse de chacun d'eux. Ces cinq centres - une fondation hospitalière à Siem Reap ainsi que deux cliniques d'Ambassades, un institut gouvernemental et un centre de soins privé, tous situés à Phnom-Penh - ont pris en charge un total de moins de 1 500 patients en 2012. Au vu des données de distribution de vaccins, environ 10 000 personnes supplémentaires ont reçu du vaccin en pré- ou en post-exposition en 2012 dans le pays.

La plupart des centres ont dit avoir recours à une PPE intramusculaire, administrant une dose complète de 0,5 mL au cours de chacune de quatre sessions. Le coût de la PPE a été estimé après des recherches sur Internet. Au vu des prix de gros, le coût moyen minimum d'une PPE intramusculaire a été estimé à 31,50 dollars US, à comparer à 12,60 dollars US pour un protocole intradermal de quatre sessions sur un mois mis au point par la Croix-Rouge Thaïlandaise. Lorsqu'il était disponible, le prix de gros d'une seule dose d'ERIG variait entre 20 $ US et 35 $ US, en fonction du fabricant.

2.4 Discussion et conclusion

Ces données recueillies par une combinaison de stratégies (extraction et analyse de données, enquête à l'aide d'un outil sur le Web, recherche de données sur Internet) ont confirmé que l'IPC était de loin le premier pourvoyeur de PPE (2/3 des traitements délivrés) au Cambodge en 2012 et qu'il était le seul à avoir recours à la PPE intradermale.

Par rapport à la première année d'exercice du centre antirabique de l'IPC, le nombre de patients consultant en 2012 était beaucoup plus élevé avec un pourcentage supérieur de patients originaires des provinces (hors de Phnom-Penh). Bien que les patients venus consulter étaient originaires de toutes les provinces à l'exception d'une seule, les bassins de population de l'ouest du Cambodge paraissaient très sous-représentés. Ceci pourrait refléter les obstacles auxquels ces dernières font face pour accéder à une PPE adéquate et en temps utile.

Le revenu d'un paysan Cambodgien en 2012 se situait entre 60 et 80 dollars US. Ceci fait que les coûts directs (vaccin, ERIG…), les coûts indirects (voyages et hébergements répétés en Capitale, notamment si le patient est un enfant accompagné d'un parent) et la perte de revenus dus à la PPE ne sont pas supportables pour les populations les plus à risque. Le raccourcissement du protocole PPE à trois sessions sur une seule semaine aiderait à réduire les coûts directs (vaccins) et indirects (transports, séjours répétés) pour les patients originaires des zones rurales et à améliorer l'équité dans l'accès à une PPE adéquate en temps utile au Cambodge.

Ce travail a été publié dans le Journal of Travel Medicine[71].

3. Partie 3: Améliorer l'accès géographique à la prévention antirabique

Une stratégie épidémiologique basée sur l'évidence pour positionner des centres périphériques de prévention de la rage de manière optimale au Cambodge

3.1 Introduction

Nous avons analysé les caractéristiques des patients documentés dans la base de l'Institut Pasteur du Cambodge (IPC) et n'ayant pas amené leur protocole de PPE à leur terme pour estimer le nombre de personnes qui bénéficieraient au mieux du positionnement d'un centre antirabique dans le voisinage de leur district de résidence.

3.2 Méthode

Les étapes successives sont résumées dans le tableau ci-dessous.

Nous avons procédé à une extraction de la base de données des patients consultant à l'IPC pour PPE au cours des années 2009-2013. Les schémas vaccinaux considérés complets étaient basés sur les recommandations OMS 2010 [11] et le protocole de la Croix Rouge Thaïlandaise. Ils étaient considérés incomplets s'ils avaient été interrompu avant la 5è sessions avant le 6 juin 2012 ou la 4è session à partir de cette date, à moins que : 1/ l'animal mordeur soit encore en vie à J_{10} (stop après la 3è session) ; 2/ le patient avait été vacciné selon les fichiers de l'IPC (stop après la seconde session de rappel) ; ou 3/ la tête du chien revenait négative (stop après la 1ère session). Nous avons examiné le nombre de patients n'ayant pas mené la PPE à son terme après une morsure par un chien potentiellement enragé comme proxy des difficultés d'accès ou le non-accès à la prophylaxie, et ceci en l'absence d'indication médicale (patient déjà vacciné, survie du chien, tête testée négative…).

Tableau 1: Stratégie analytique résumée pour identifier des zones mal desservies par la PPE au Cambodge

Etape/Objectif	Méthode
1. Constitution de la base de données	• Extraction pour les années 2009-2013 de la base de données patients PPE du centre antirabique de l'Institut Pasteur du Cambodge (IPC) ; • Exclusion des patients n'ayant pas mené la PPE à terme en raison de recommandations médicales (chien testé négatif ou ayant survécu, vaccination antérieure…) ;
2. Recueil de données reflétant les difficultés d'accès à l'IPC	• 159 centres de santé de district du pays contactés ; • Documentation: – de la distance de Phnom-Penh: distance euclidienne, distance par la route, en minutes de trajet, en termes financiers (coûts de transport aller-retour) ; – des mois correspondant aux inondations et à la période de la récolte du riz.
3. Incomplétude PPE et facteurs non-géographiques	• Identification des patients à schémas PPE non complétés malgré les recommandations. • Analyse univariée puis régression logistique multivariée afin d'identifier tous les facteurs non géographiques associés à une incomplétude de la PPE (<5 sessions jusqu'à Juin 2012; <4 sessions après cette date). • Calcul d'un odds ratio ajusté d'incomplétude pour chaque facteur non-géographique.
4. Incomplétude PPE et district de résidence	• Modèle logistique multivarié explorant l'association entre le district de résidence et la complétude vaccinale, après ajustement pour les facteurs non-géographiques identifiés à l'étape précédente ; • Calcul d'un odds ratio ajusté d'incomplétude pour chaque district.
5. Calcul du nombre attendu de PPE incomplètes pour chaque district de résidence	• Calcul basé sur l'odds ratio d'incomplétude (Etape 4) permettant une approximation du risque relatif[105,106]. • Calcul du pourcentage de risque attribuable[107] d'incomplétude; • Multiplication de ce risque attribuable d'incomplétude par le nombre estimé de personnes mordues par des chiens dans chaque district. • Produit le nombre absolu de personnes ne complétant pas le protocole PPE théoriquement évité par le positionnement d'un centre antirabique dans ce district. $RI_{district\ (i)} = ARP_{ex} * N_{bitten} = ((RR - 1) / RR) * (incid_{bites} * pop_{district})$ *Où ARP_{ex} = pourcentage de risque attribuable parmi les exposés; N_{bitten} = nombre de personnes mordues par des chiens attendu chaque année dans le district; RR = risque relatif; $incid_{bites}$ = incidence annuelle de morsures de chien, appliqué à ce district [67]; $pop_{district}$ = population du district en 2008* [108]
6. Identification d'un seuil de distance associé à l'incomplétude	• Modèle par arbre de décision (« boosted regression tree ») explorant le rôle du trajet du centre du district (documenté à l'Etape 2) jusqu'à Phnom-Penh dans l'incomplétude PPE, après ajustement pour les facteurs non-géographiques associés à l'incomplétude (Etape 3). • Identification d'une distance-seuil à partir de laquelle l'incomplétude augmente de manière significative.
7. Identification de zones mal desservies	• Cartographie du Rabies Index pour chaque district de chaque province, en attribuant la valeur 0 : – à Phnom-Penh (site de l'IPC); – à six provinces distantes et faiblement peuplées contribuant ≤ 5 patients à l'étude; – à tous les districts situés en-deçà du seuil de distance significativement associé à l'incomplétude vaccinale (Etape 6) • Analyse via la méthode de continuité des polygones[109] et la statistique Gi* de Getis et Ord[110] pour identifier des agrégats de districts caractérisés par un indice de rage élevé (clusters d'incomplétude élevée).

L'association de divers facteurs avec l'incomplétude vaccinale a été étudiée à l'aide de modèles univariés et de modèles multivariés de régression logistique. En outre, un modèle d'arbre de régression boosté a été utilisé pour tenir compte d'associations non-linéaires impliquant les caractéristiques de la distance entre le district de résidence et l'IPC.

Quel que soit le modèle utilisé (régression logistique ou arbre de régression boostée), la qualité de la prédiction de la probabilité d'incomplétude a été estimée par l'aire sous la courbe receveur-opérateur (ROC) associée à cette prédiction.

Le pourcentage de risque attribuable (« risque attribuable »)[107] a été calculé à l'aide du risque relatif – lui-même approché par le rapport de cotes (odds ratio, OR) d'incomplétude associé à chaque district, produit par le modèle[105,106]. Ce risque attribuable a ensuite été multiplié par la

population de chacun des districts et par l'incidence estimée de morsures de chien[67] pour obtenir une mesure d'impact dans la population, dénommée Rabies Index (RI). Ceci représente le nombre absolu de protocoles de PPE incomplets qui seraient théoriquement évités par l'établissement d'un centre de prévention de la rage dans ce district. Les RI de chaque district ont ensuite été cartographiés et la méthode de conceptualisation de la Continuité des Polygones[109] puis la statistique Gi* de Getis et Ord[110] a été calculée. Ceci a permis d'identifier non seulement les districts présentant un nombre élevé de PPE incomplètes mais des agrégats (clusters) de districts à RI élevés, dans le voisinage desquels l'établissement d'un centre périphérique de prévention de la rage devrait selon toute vraisemblance avoir le plus d'impact. Les Districts contribuant ≤5 patients à la base ont été exclus, car le nombre attendu de PPE incomplètes était ≤ 1.

3.3 Résultats

Pour la période de 2009 à 2013 inclus, les 100 660 patients inclus étaient de sexe masculin dans 52% des cas avec un âge moyen de 21 ans (écart type 18,7 ans) et une médiane à 13 ans (IQR 6-32). Les patients provenaient de 18 des 24 provinces du Cambodge.

Le schéma vaccinal PPE a été considéré incomplet dans 7 814 (7,8%) des cas. Le risque d'incomplétude PPE était plus élevé pendant la période de la récolte de riz, a augmenté au fil des années, était plus élevé après l'année 2010, lorsque le délai de consultation dépassait deux jours, quand le patient était un adulte âgé de 15 à 49 ans, lorsque le chien avait été abattu ou été perdu de vue, ou enfin lorsque le schéma prescrit était un schéma complet (de 4 à 5 sessions selon les années). Le risque d'incomplétude PPE était moindre lorsque les patients étaient de sexe féminin ou lorsque le chien mordeur semblait malade ou était confirmé enragé. Certains districts étaient associés à une incomplétude particulièrement élevée comme dans la province distante et faiblement peuplée de Preah Vihear, mais aussi à l'inverse à une incomplétude faible dans des districts de la province de Kandal, voisine de Phnom-Penh. Parmi les différents modèles évalués, celui basé sur la distance par la route en kilomètres avait l'aire sous la courbe ROC la plus élevée (0,883). Ceci nous a permis d'identifier un effet-seuil associé à une augmentation marquée de l'incomplétude, pour une distance parcourue d'environ 150 km. Après calcul du RI à partir des odds ratio significatifs de chaque district obtenu précédemment, la cartographie des groupes de districts a identifié deux grands bassins dont la population bénéficierait du positionnement plus proche de centres antirabiques, dans les villes de Battambang à l'ouest, près de la Thaïlande et de Prey Veng sur la frontière avec le Vietnam.

3.4 Discussion et conclusion

En résumé, l'incomplétude vaccinale dans cette très importante base de données était d'environ 8%, et augmentait très significativement pendant la période de la récolte de riz, moment où les Cambodgiens doivent faire le choix cornélien entre la préservation de leur santé face au risque rabique et la subsistance de leur famille pour l'année à venir. Certains districts sont en soi associés à un risque très élevé de non complétude, avec un seuil très marqué à partir d'une distance d'un centre antirabique d'environ 150 km parcourus par la route.

Pour une estimation plus précise de l'efficacité du positionnement de deux centres périphériques de vaccination contre la rage au Cambodge, nous nous sommes donc basés non pas sur une mesure du risque mais sur une mesure d'impact en extrapolant ce risque d'incomplétude à toutes les populations, y compris celles des personnes n'ayant pas consulté.

Enfin le résultat final de notre étude est une carte d'agrégats de districts où le nombre théorique de personnes qui compléteraient leur schéma vaccinal si un centre y était positionné serait le plus élevé. Les habitants du plus gros cluster à l'est de Battambang n'auront plus qu'à parcourir 60 km environ au lieu de 180 km, et ceux du cluster proche du cercle 120 km au lieu de 150. Idem pour les patients de Prey Veng, qui iront à Kampong Cham à 110 km au lieu de Phnom Penh à 150 km. Les provinces de Battambang et les provinces voisines (Pailin, Pursat, Siem Reap, Banteay Meanchey) avaient une population totale de 2 041 962 (15,2%) sur 13 395 682 habitants lors du dernier recensement au Cambodge en 2008[108].

Ce travail a été publié dans l'American Journal of Epidemiology[111].

4. Partie 4: Améliorer l'accès financier à la prévention antirabique

Une description clinique et épidémiologique d'une base de données observationnelle parmi les patients ayant abrégé leur PPE

4.1 Introduction

La PPE ne serait actuellement accessible qu'à moins de 5% des personnes mordues par un chien. Outre la méconnaissance de la conduite à tenir, l'obstacle majeur au Cambodge est d'abord la difficulté d'accéder à la PPE, qui n'est disponible pour le plus grand nombre qu'au niveau de la capitale[71]. Ce problème d'accès géographique a été abordé dans notre précédent travail[111]. Reste l'obstacle majeur de l'accès financier dans un pays où le revenu mensuel par habitant avoisine les 40 USD. Le raccourcissement de la PPE sur une semaine au lieu d'un mois pourrait

avoir un impact majeur sur l'accès des populations rurales Cambodgiennes à la PPE. Nous avons donc cherché à documenter l'efficacité clinique de la PPE lorsqu'elle avait été interrompue par les patients après 3 sessions par rapport à la PPE complète de 4 sessions ou plus chez les patients ayant consulté au centre antirabique de l'Institut Pasteur du Cambodge entre 2003 et 2014 inclus.

4.2 Méthode

Les étapes successives de la méthode sont détaillées dans le tableau ci-dessous.

Tableau 2: Stratégie analytique résumée pour documenter l'association entre la prophylaxie post-exposition (PPE) abrégée (3-sessions) et la survenue d'une mort par rage probable.

Etape/Objectif	Méthode
1. Constitution de la base de données	• Extraction des données 2003-2014 à partir de la base de données des prophylaxies post-exposition (PPE) de l'Institut Pasteur du Cambodge (IPC). • Exclusion des patients: – Antérieurement vaccinés contre la rage; – Mordus par des chiens testés négatifs ou des chiens non-testés ayant survécu (pas d'exposition à la rage).
2. Rappel des patients ≥6 mois après la PPE	• Rappel du numéro de portable renseigné dans la base de données. • Si échec, contact du centre de santé du District de résidence du patient puis du Chef de village de résidence puis des patients (en Khmer) afin de: – Confirmer l'identité du patient; – Obtenir leur consentement éclairé; – Documenter l'évolution Clinique. • Si le patient était inconnu du Chef de village, le patient était considéré perdu de vue.
3. Identification des morts par rage	• Si le patient était identifié mais était décédé. • Autopsie verbale par un médecin Khmerophone connaissant la rage (entretien semi-structuré des proches, à l'aide d'un questionnaire standardisé). • Détermination de la cause la plus probable du décès (rage ou autre cause) par un comité d'experts externe, ignorant nos conclusions.
Performance de la PPE, globale et par catégorie de nombre de sessions reçues	
4. Morts de rage: Total et par nombre de sessions PPE reçues	• Pourcentage global de mort par rage parmi les personnes mordues par un chien confirmé enragé et par un chien confirmé enrage ou d'aspect malade mais non testé, avec intervalle de confiance binomial exact. • Nombre de décès par rage, stratifié par: – Nombre de sessions de PPE; – Traitement par RIG; – Statut du chien mordeur.
5. Test des associations entre le nombre de sessions PPE reçues et la mort par rage	• Estimation de la probabilité qu'un décès précoce soit alloué au groupe 3 sessions par: – La proportion observée des patients de la base ayant reçu 3 sessions, en excluant les décès précoces ; – Régression logistique multivariée pour documenter l'association entre le nombre de sessions reçues et toutes les caractéristiques documentées dans la base de données initiale pour calculer la probabilité à l'aide des coefficients. • Calcul du Test unilatéral de Fisher et valeur mid-point p pour chaque hypothèse d'allocation des décès ; • Test unilatéral de Fisher pondéré et valeur mid-point p, en tenant compte de l'incertitude de l'allocation des morts par rage survenues entre la 3è et la 4è session (morts précoces) dans les catégories de nombre de sessions reçues.
Le travail ne porte ensuite que sur l'hypothèse la plus probable d'allocation des décès précoces par rage	
6. Détermination des caractéristiques de l'association entre le décès par rage et le nombre de sessions PPE	• Calcul de l'odds ratio inconditionnel estimé par la maximisation de la vraisemblance et obtention de l'intervalle de confiance à 95% du mid-point p ajusté obtenu en inversant le test de Fisher. • Estimation de la puissance du test de Fisher unilatéral pour un odds ratio théorique proche de la valeur estimée.
7. Association d'autres facteurs que le nombre de sessions PPE avec la survenue d'une rage	• Régression logistique univariée et multivariée des caractéristiques des patients dans la base de données pour estimer l'association entre complétude de la PPE (3 *vs.* 4+ sessions) et la survenue d'une mort par rage après ajustement sur tous les autres facteurs.

Les données sociodémographiques, relatives aux lésions et au statut du chien concernant les patients pour la période 2003-2014, inclus, ont été extraites de la base documentant les consultations successives des patients mordus depuis 1998, ainsi que le résultat des tests virologiques par immunofluorescence effectués sur les têtes de chien[59].

Tous les patients mordus par des chiens confirmés enragés ou malades mais non testés étaient éligibles, à l'exception des patients déjà vaccinés, de ceux mordus par des chiens testés négatifs pour la rage ou ayant survécu (donc non enragés). Chacun des patients a été contacté par téléphone après un délai d'au moins 6 mois, en utilisant d'abord le numéro de portable renseigné dans la base de données. Les Cambodgiens changeant souvent de carte SIM et ne pouvant être joints par courrier, le centre de santé du district de résidence de chaque patient non joignable a été contacté afin d'obtenir le numéro de portable du Chef de village. L'appel du Chef de village a permis à l'équipe de vérifier qu'une personne du nom du patient résidait dans le village et, si c'était le cas, d'obtenir son numéro ou de convenir d'un rendez-vous téléphonique. C'est au cours de ce rendez-vous, mené en Khmer, que l'identité du patient était confirmée, que le consentement à participer était obtenu et que l'évolution clinique était documentée auprès des patients ou de leur famille. Les patients qui ne répondaient pas au numéro renseigné dans la base ou qui ne pouvaient être identifiés par le Chef de village étaient considérés perdus de vue. Les résidents de Phnom Penh ont été exclus de l'étude, les premières semaines ayant montré que ceux-ci étaient très mobiles donc impossibles à retracer.

En cas de décès du patient, une autopsie verbale était menée par téléphone avec les proches par un médecin Khmérophone ayant l'expérience des cas de rage, lors d'un entretien semi-structuré à l'aide d'un questionnaire standardisé. Ces rapports d'autopsie verbale ont tous été revus par un groupe d'experts extérieurs n'ayant pas connaissance de nos propres conclusions pour attribuer la cause probable de décès à la rage ou à une autre pathologie.

Le pourcentage global de décès par rage malgré la PPE parmi les personnes mordues par un chien enragé ou par un chien d'aspect malade mais non testé a été calculé, ainsi que l'intervalle de confiance exact. L'analyse a ensuite porté sur tous les patients pour estimer le pourcentage de décès par rage selon le nombre de sessions vaccinales lors de la PPE, selon l'administration ou non de RIG et selon le statut du chien. Les morts précoces - après la 3è session mais avant la 4è session - ont fait l'objet d'une démarche particulière pour évaluer la probabilité qu'aurait été la leur de compléter le schéma PPE, sur la base de: 1/ la proportion des PPE n'ayant pas dépassé la 3è session observée dans la base de données (après exclusion des patients morts précocement de la rage) et 2/ la probabilité de chaque patient décédé précocement de la rage d'appartenir au groupe de patients ayant reçu 3 sessions seulement, estimée par une régression logistique

multivariée de l'appartenance à un des deux groupes (3 vs. 4+ sessions) comme fonction de toutes les caractéristiques des patients documentées dans la base de données.

La valeur mid-p du test de Fisher unilatéral testant l'association entre l'incomplétude de la PPE et la survenue d'un décès par rage a été calculée pour chaque série possible d'allocation des décès précoces par rage (par exemple 0, 1, 2, … , tous les décès précoces alloués au groupe 3 sessions, les autres décès précoces par rage étant alloués au groupe 4+ sessions). La valeur de mid-p du Fisher unilatéral a ensuite été obtenue en multipliant la valeur mid-p du Fisher unilatéral de chaque hypothèse d'allocation par la probabilité de survenue de cette hypothèse, comme décrit ci-dessus.

Les analyses ont ensuite été restreintes à l'hypothèse d'allocation des décès par rage la plus probable. L'odds ratio inconditionnel a été estimé par la maximisation de la vraisemblance et l'intervalle de confiance à 95% du mid-p ajusté a été obtenu en inversant le test[112]. La puissance du test du mid-point ajusté a été estimée *a posteriori*, sur la base d'un odds ratio ayant une valeur théorique proche de celle estimée. Un modèle de régression logistique a ensuite exploré l'association entre divers facteurs et la survenue d'un décès par rage, et une autre l'association entre le fait de recevoir 3 ou 4+ sessions PPE et la survenue d'un décès par rage. Un seuil de significativité statistique de 5% a été retenu pour tous les tests.

4.3 Résultats

Au total, 3 838 patients ont reçu une PPE par vaccin préparé sur cellules Vero entre 2003 et 2014 après morsure par un chien confirmé enragé ou non testé mais d'aspect malade.

Après exclusion de 520 résidents de Phnom Penh et 513 patients perdus de vue, l'échantillon final était de 1 739 patients mordus par des chiens enragés et 1 066 patients mordus par des chiens non testés mais d'aspect malade, soit 2 805 patients au total. L'enquête a permis d'identifier 24 décès de cause autre que la rage (accidents de la voie publique, noyade, douleur thoraciques, ascite, etc.) dont deux décédés avant le terme de six mois après PPE, et trois morts de la rage. Le pourcentage de décès par rage après morsure par un chien enragé malgré la PPE est donc de 3/1 739 soit 1,7 pour 1 000. Il est de 3/2 805 soit 1 p. 1 000 après morsure par chien enragé ou suspect, malgré la PPE.

Deux des trois décès attribués à la rage étaient des décès précoces. La valeur mid-point p du test de Fisher unilatéral pondéré était de 0,0959. Après la prise en compte des probabilités individuelles dérivées du modèle de régression logistique pour que les deux décès précoces appartiennent au groupe 3 sessions, la valeur mid-point p du test de Fisher pondéré est de 0.0961.

En analyse univariée, les morsures de Catégorie III et le fait d'être mordu principalement à la tête / au cou étaient associés à un risque plus élevé de rage. Les trois décès par rage sont tous survenus après une morsure de Catégorie III. Par conséquent, aucun modèle de régression logistique multivarié n'a pu être développé (pas de convergence atteinte), rendant impossible l'ajustement sur des variables indépendantes.

L'odds ratio avant ajustement de l'association entre le fait de mourir de la rage et le fait de ne recevoir que 3 sessions était de 6,30 mais l'intervalle de confiance incluait 1. Il était moindre - à 4,44 - mais également non significatif chez les patients mordus par des chiens non testés. Cependant, la puissance du Fisher unilatéral chez les patients mordus par des chiens confirmés enragés a été estimée à 49%.

4.4 Discussion et conclusion

En résumé, 99,83% des patients mordus par un chien enragé n'étaient pas morts de la rage avec un suivi d'au moins 6 mois après la PPE. L'étude a conclu qu'il n'y avait pas d'élément en faveur d'une différence du pourcentage de décès parmi ceux ayant reçu trois sessions de PPE par rapport à ceux ayant reçu 4 sessions ou plus. La puissance, à 49%, impose cependant une certaine prudence durant l'implémentation de schéma PPE abrégé.

Cet article a été soumis en Août 2018 à la revue *Vaccine*.

5. Partie 5: Suites et perspectives

Les travaux résumés ici ont d'ores et déjà été suivis d'effet et pourraient avoir une portée significative, au Cambodge et ailleurs.

A la suite du travail d'identification de zones mal desservies, le ministère de la santé du Cambodge a décidé la mise en place de deux centres périphériques de vaccination dans deux villes de province, Battambang à l'ouest et à Kampong Cham à l'est du pays et qui est localisé au niveau d'un nœud routier situé non loin de Prey Veng. L'IPC travaille actuellement à la mise en place du premier de ces centres pour desservir l'ouest du pays.

Au moins 30 M de personnes reçoivent une PPE à travers le Monde chaque année. L'adoption d'un protocole PPE intradermal abrégé de 3 sessions sur une semaine pourrait permettre une meilleure équité en soignant 33% de patients en plus pour un stock de vaccin antirabique disponible, de réduire les coûts institutionnels et les coûts directs et indirects pour les patients. Une étude de modélisation vient d'être soumise pour publication, qui montre que ce schéma de

PPE de 3 sessions sur une semaine a le ratio coût: efficacité le plus élevé des schémas actuellement proposés.

C'est compte-tenu de ces éléments que les nouvelles recommandations d'un groupe de travail du SAGE entérinées par le SAGE ont été publiées officiellement par l'OMS[113,114]. Le 20 Avril 2018, l'OMS a proposé que ce protocole PPE – le premier protocole sur une semaine et dénommé « protocole IPC » - soit adopté à travers le Monde. Dans tous les cas, l'introduction de ce protocole doit s'accompagner du renforcement de la pharmacovigilance et du rappel des patients. Ceci sera prochainement examiné par le Gavi[115], qui devrait assouplir sa position et accepter de soutenir financièrement la PPE sur une semaine dans 47 pays éligibles.

PhD dissertation in English

Summary

Rabies causes more than 60,000 deaths worldwide each year, including 800 in Cambodia, where canine-mediated rabies virus circulates.

Death occurs in nearly 100% of rabies cases, a disease which is nearly 100% avoidable by timely and adequate rabies post-exposure prophylaxis (PEP). Improving access to PEP in rural areas of endemic countries will spare human lives in the short term.

This epidemiology PhD used the data collected in patients referred to the rabies prevention clinic and tested dogs at Institut Pasteur du Cambodge (IPC), Phnom Penh. After a baseline assessment of access to and obstacles to access timely and adequate PEP in Cambodia, this PhD aims to contribute to improving: 1/ geographical access and 2/ financial access to PEP for rural populations in Cambodia.

We developed an original strategy to identify populations with a high risk of PEP noncompletion after a bite by a potentially rabid dog. This should help improve geographical access to PEP following the implementation in July 2018 of a peripheral rabies prevention center in Western Cambodia. This strategy can be applied to identify difficulties in accessing health services relevant to other health issues, under certain conditions.

After patient callback and analysis of rabies deaths among those who did and did not complete the 4-sessions/1-month intradermal PEP regimen of their own accord, we were unable to demonstrate a difference in rabies mortality among patients who only received 3 vaccine sessions over the first week compared to those receiving at least 4 sessions/one month. Abridging the protocol to one week would reduce direct and indirect costs and the loss of income during PEP in the Capital. The adoption of this abridged regimen must be associated with a strengthened clinical monitoring system for at least 6 months following patients' initial PEP.

The work presented in this PhD has implications which reach beyond Cambodia: WHO recommends this new IPC regimen – the first approved one-week, abridged rabies PEP regimen – in its April 2018 guidelines.

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Preamble

Few more than a hundred people die from rabies each year in France. But the moving sight of a rabies patient, the certainty of impending death, the anguish of the long incubation period suffice to explain the terror it inspires. Its obscure cause, the variability of its incubation time, the absence of anatomical lesions capable of explaining symptoms in animal or human rabies cases have, so to speak, stimulated the curiosity of medical doctors and veterinarians, and written contributions on this issue can be counted by the hundreds. When we read this abundant literature, we are surprised to see how often findings are contradictory and we are amazed at how few important points remain definitively documented after so much effort.

Author's transl. from Emile Roux: Des nouvelles acquisitions sur la rage.

Thèse de Médecine. Paris; 1883

Rabies is the zoonosis longest and best known to humankind. It is also the human disease with the highest case-fatality rate when clinical signs appear. It may be the most feared for its unpredictability, its suddenness and perhaps because of our fear of returning to an uncontrolled, animalistic state, becoming - in effect - "barking mad"[116].

Technical advances have been many over the past 50 years and are communicated, but often only in the scientific literature. How then can the necessary “knowledge translation” be performed, extrapolating from data on the inoculation of rabies virus into the footpads of raccoons to evaluate risk in a bitten child in routine clinical care?

Although much has changed in the comprehension of the causes and in the primary and the secondary prevention of rabies, and despite progress in active or compassionate management of encephalitic patients[117,118], 21st-Century clinicians remain as incapable today of curing clinically-declared rabies as they were 4,000 years ago[1] (see Annex Chapter 1). While we wait for progress to be made in the identification of antiviral agents capable of eliminating the virus, preventing rabies deaths must - for the time being - rely on effective primary prevention of animal bites through responsible dog ownership, primary canine vaccination and secondary (post-exposure) prophylaxis in bite victims, all delineated in the 19th Century.

The Institut Pasteur du Cambodge (IPC), Phnom Penh, is Cambodia's largest rabies post-exposure referral center, with over 22,000 referrals in 2015. IPC also houses the Reference center for rabies diagnosis in the Virology Unit - with access to state-of-the-art techniques, a BSL3 laboratory and access to further expertise in the Pasteur Network - and a highly capable epidemiology team with a recognized clinical research group on a single campus. This alliance

provides a unique and formidable frontline tool in a highly endemic setting to undertake innovative rabies research in animal heads and among patients exposed to confirmed rabid dogs.

Over the past century, efforts have been made to shorten and simplify rabies vaccination protocols, with an evidence base stemmed in careful scientific and medical research. Medical research is often difficult, but medical research on rabies is like no other: This is due to issues linked with the developing setting and with this lethal and terrifying disease itself, which severely limit possible methodological approaches.

In Cambodia - as in many developing rabies-endemic countries - there are no systems for veterinary quarantine or surveillance of rabies. Dogs who have bitten escape or are immediately slain, often being used for food. Virological confirmation of animal rabies is therefore rare. Although Cambodia suffers one of the greatest burden of (constantly fatal) rabies *per capita* worldwide, human rabies remains infrequent. Rabies remains a mainly rural disease. Cambodians - especially in the rural setting - have difficulty accessing either information or vaccine for rabies prevention, whether primary or secondary. Clinicians do not always establish a clinical diagnosis; Obtaining, transporting and preserving brain samples can be a challenge. "Paralytic" forms of rabies lead to underestimation, especially in the tropical setting where differential diagnoses for encephalitis are rife. As rabies is the disease with the highest case-fatality rate - even if managed in intensive care units - vaccine studies against placebo are inconceivable. Experimental deviation from existing vaccine protocols in suspected - let alone confirmed - rabies exposure may be perilous. Diagnosis of rabies infection in the CNS cannot easily be performed *intravitam*: Even in case of inoculation and infection, active replication of rabies virus takes place in unattainable body compartments[59.] No *post-hoc* serological studies are possible as humans do not survive clinical rabies, especially in developing countries. Postmortem diagnosis is also difficult as families refuse autopsy due to major issues with culture and funeral rites. Effectiveness of prevention and treatment among rabies-exposed humans are therefore impossible to assess in real time.

But there are trump cards.

The date of the bite exposure is usually known in rabies. The prolonged incubation period allows for beating the virus in a race to the death. Vaccine or protocol efficacy can therefore be assessed by documenting survival. Although inconstant in rabies cases, “hydrophobia” is a striking and tell-tale sign which has become synonymous with rabies. Its clinical description leads to a highly probable rabies diagnosis if associated with a documented dog bite, giving verbal autopsy techniques near 100% sensitivity and specificity when hydrophobia is present. In

Cambodia as in many other developing settings, rabies (ជំងឺឆ្កែឆ្កួត - "Chum ngeu chkae chkuot") is a matter of grave concern to the population, widespread and well identified. With an incidence comparable to road deaths in France, every Cambodian knows someone - or knows someone who knows someone - who died with hydrophobia after a dog bite. In an unpublished study conducted by L. Chiffot at IPC in 2013, rabies was the only zoonosis (with bird flu) invariably identified by people in 12 villages in Mondulkiri and Sihanouk Provinces. Incubation before symptomatic complications is short compared to some other infectious diseases such as hepatitis or HIV, and vaccine effectiveness can be clinically assessed within a few weeks or months. The literature on rabies is ancient and abundant, dating back to before the advent of post-exposure prophylaxis, and provides precious pre-vaccine clinical, epidemiological and other scientific data. There are many reference documents on prevention and management at the individual and collective level of animal and human rabies. Unlike other some other diseases linked to sexual mores or lack of governance, rabies is not considered a "shameful disease" and can readily be addressed by authorities. It is high on the list of priority zoonoses for research in Cambodia, which has endorsed and committed to the ASEAN strategy of canine rabies elimination by 2030[100,119].

The characteristics of various aspects of rabies mentioned above profoundly impact epidemiological research strategies. As rabies remains relatively infrequent, population cohorts are unwieldy. Case-control studies, however, are definitely an option. Syndromic surveillance may be reliable only with furious rabies but can readily be undertaken in hospitals or in the community. Patients can be contacted six months after receiving PEP to assess vaccine efficacy. If referred patients have died, verbal autopsies can reliably be used to differentiate rabies from other deaths[51,63–65]. However unpalatable, viral challenge and PEP experiments can - if necessary - be undertaken in laboratory animals to spare human lives. Post-vaccination serological studies can be conducted using recommended protocols and at no risk to humans volunteers or patients bitten by a potentially or confirmed rabies-infected dog. Finally, as the database at IPC is well-maintained with nearly 275,000 fully documented PEP referrals, *in natura* ("natural") experiments in patients who did not complete PEP protocols for personal reasons or reasons of financial or geographical access present a unique opportunity to assess clinical outcome in patients with real-life exposure who received shorter than recommended regimens.

While many strive to prove not the concept but the real-life operational significance of actionable One-Medicine/One-Health in the developing setting, this PhD thesis will make use of epidemiological approaches and clinical, virological and surveillance data gathered by research

teams at Institut Pasteur du Cambodge to spare human lives by improving geographic and financial accessibility to rabies post-exposure prophylaxis, in Cambodia and beyond.

Figure 1: Portrait of Emile Roux (1853-1933) ca. 1889
(© Institut Pasteur – Musée Pasteur)

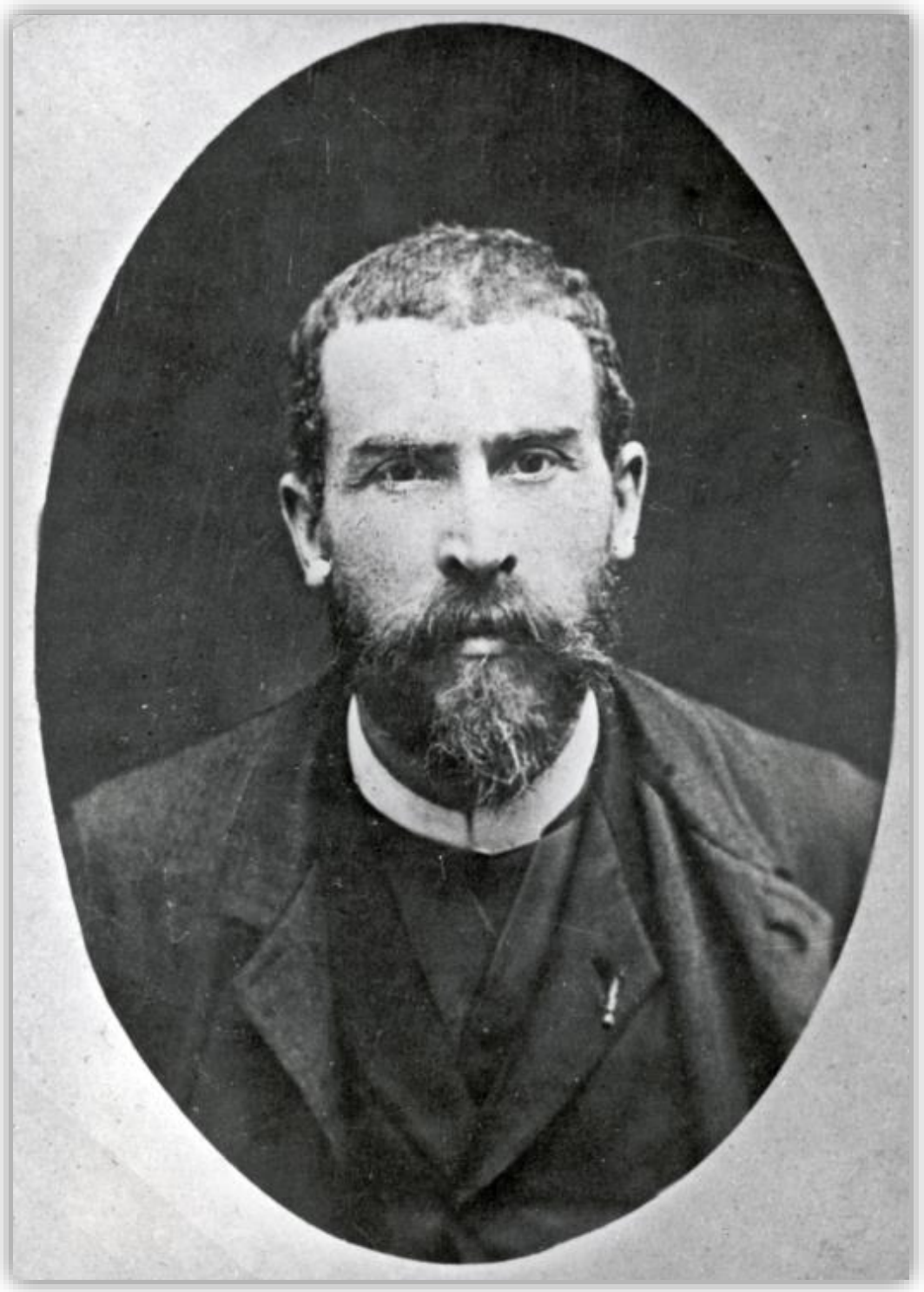

Figure 2: Albert Edelfelt (1854-1905): Louis Pasteur (1885); Oil on canvas (© RMN-Grand Palais (Musée d'Orsay) / DR), showing Pasteur examining a desiccated spinal cord from a rabbit experimentally infected with rabies.

Part 1: Background and rationale

1. Vaccines and vaccination against rabies

1.1 Rabies vaccines in humans

1.1.1 Nerve-tissue vaccines

In 1911, D. Semple and his team furthered the Pasteur team's seminal vaccine[2,8,120–122] work using phenol-inactivated (killed) rabies virus cultivated on sheep's or goats' brain tissue (see historical review in Annex Chapter 1). The effectiveness of this vaccine was estimated to be 50% to 84%[124] in rabies-exposed patients. Nerve tissue vaccines (NTV) such as Semple/Fermi-type or later Fuenzalida-Palacios vaccines produced using various preparations[9] were then used for several decades across the World, saving countless lives[10,125–127].

These were, however, associated with severe side effects due to their animal myelin content. The World Health Organization has recommended the discontinuation of nerve-tissue vaccine use (and production) since 1983[61,128]. These were progressively phased out worldwide. In Asia, Pakistan and Myanmar were consuming remaining stocks and aimed to phase out NTVs by the end of 2015. Nerve-tissue vaccines currently remain in use/production in Algeria, Argentina, Bolivia and Ethiopia to December, 2017.

1.1.2 Cell-culture and embryonated egg vaccines

Nerve-tissue vaccines then purified NTV were followed by the advent of modern cell-culture and embryonated egg vaccines (CCEEV) still in use today[11,14,129,130]. These vaccines based on techniques developed in the late 1950s do not require animals to be infected and are more antigenic and better tolerated[11,13,14], with an estimated neurological complication rate not above the background rate of about 1 per 100,000 per year[131]. Furthermore, the industrial processes are qualitatively and quantitatively better suited for large-scale and uninterrupted production and supply (although vaccine supply regularly continue to be interrupted at the international level)[13,132]. CCEEVs are based on the viral glycoprotein (G) of inactivated RABV virus harvested from cultures of embryonic fibroblast cells (human diploid cell culture vaccine or HDCV), fetal rhesus cells, monkey kidney (Vero) cells, chick embryo cells or embryonated duck eggs[11,12]. The strain generally used for human vaccines remains the Pitman-Moore canine RABV strain[14].

The cell-culture vaccines (CCVs) now in use must in theory all meet the WHO criteria, including antigenicity (≥2.5 IU per IM dose) as determined by the mouse protection potency test) based on the National Institutes of Health (NIH) test consisting of vaccinating mice before challenging them with RABV[78,133,134]. They must also be shown noninferior to the reference human diploid cell vaccine[135]. All are produced in single IM doses[11]. Partly because of the need to ultracentrifuge low-yield cultures to produce enough RABV, their cost, however, is greater than that of NTVs and remains prohibitively expensive for many endemic countries.

To 16 August 2017, a total of 17 cell-based vaccines were produced worldwide (9 in China); To 15 January 2018 only two of these commercially-available rabies vaccines are WHO-prequalified for human use[136].

1.2 Immune response after rabies vaccination in humans

The goal of vaccination is to produce (preferably long-term) protective immunity in the overwhelming majority of vaccinees at the lowest possible risk of unwanted effects, especially severe. Whole, inactivated RABV virus vaccines are amongst the most highly immunogenic vaccines available, triggering an immune response which depends on the integrity of the G protein in the vaccine[13,84,129]. Immunity against RABV is based on T-lymphocyte- and B-lymphocyte-mediated response, which cannot be ascertained directly outside the experimental setting[129]. This response produces antibodies, which can be titrated or measured using RFITT (neutralizing antibodies) or ELISA as a proxy for protection[137].

In humans, there is a measurable and significant rise in immunoglobulin (Ig) M after one week and a rise in IgG and IgA after two weeks following rabies vaccine injection[138]. No level of antibodies is formally proven to be fully protective, but WHO experts consider an antibody level of 0.5 IU/mL as protective[11,129]. This threshold - which corresponds to complete virus neutralization after 1:50 serum dilution - is used as a standard by which to confirm protection in personnel requiring immunization to carry out their duties (veterinarians, researchers...)[139].

In the overwhelming majority of published studies, 100% of participants receiving cell-based vaccines presented neutralizing antibody titres above 0.5 IU/ml by Day 14[86–94], whether with Vero cell-based vaccine[80] or Purified chick embryo cell-culture vaccine[140] and whatever the age[141] and nutritional status[85,142], when documented. This concludes to equivalent clinical protection by PVRV or PCECV by Day 14[86–94], including in studies comparing the two types of vaccine for pre-exposure[143] or post-exposure[85] prophylaxis. In immunocompetent vaccinees, post-vaccine immunity lasts decades and the anamnestic response is extremely robust, including after low-dose intradermal administration[129,139,144–148]. Even after immunity has waned below detectable levels, an intramuscular or intradermal booster shot

or exposure to the virus causes a rapid and long-term rise in titers and protection from viral challenge, even in cases of high RABV inocula such as organ transplant[129,149]. Boosters are recommended to stimulate immunity in case of renewed exposure, but only one rabies case has been reported after unsuccessful pre-exposure vaccination in a person receiving chloroquine[129,150,151]. In clinical practice, a person who has been identifiably protected once is now considered protected against rabies for life.

1.3 Issues impacting efficacy

Rabies PEP was "ineffective" in terms of virus neutralizing antibody detection in some patients with unchecked HIV infection or AIDS, with advanced B-cell lymphoma or kidney transplant[152]. A publication from Iran showed that an Essen intramuscular protocol using Vero cell vaccine had 100% immunological effectiveness in 50 patients with various comorbidities or pregnancy[153]. Whether or not pregnancy interferes with antibody production to some extent, bite victims who received PEP while pregnant nevertheless overwhelmingly survived potential or confirmed rabies exposure (see section below)[11,154]. Administration of high doses of RIG reduces antibody response to HDCV, leading to the determination of a maximum RIG dose per kilo weight[143,155]. Concurrent medication other than immunosuppressants may also have an impact on vaccine effectiveness: Chloroquine has been shown to inhibit response to rabies vaccination *in vitro*[156,157] and *in vivo* after pre-exposure prophylaxis[150,158,159]. Whether that effect is shared by other chloroquine combination drugs or other 4-aminoquinolines derivatives is unclear. Although there is no clinical evidence to support this, the intramuscular route is thought by some to be more indicated than intradermal administration in cases such as these[61,160]. The solution may lie rather with intradermal dose volume or repetition.

The impact of sex, age or body mass index on the speed and quality of the neutralizing antibody response to intradermal rabies vaccination has been documented by the ongoing RESIST-2 study at Institut Pasteur du Cambodge. Intermediate results so far show no clinically relevant difference in antibody response according to age, sex, or whether patients were underweight, overweight, or in between the two.

1.4 Rabies vaccination risks

Aside from local and benign effects, modern rabies vaccines are extremely well tolerated[80,131,161]. The availability of several types of highly antigenic vaccines allows persons to be immunized even in case of allergy to egg proteins[162]. Although all available data point to its innocuousness when administered after a potential rabies exposure in pregnant women[163–169], it is currently recommended that pre-exposure vaccination be deferred in case of pregnancy. This recommendation, however, is to change in the short-term.

1.5 Cross-protection

In experimental studies, RABV vaccination has been found to be cross-protective with European and Australian Bat Lyssaviruses and some Asian lyssaviruses belonging to Phylogroup I[170–172]. Protection against Phylogroup II, III and IV lyssaviruses (see virological review in Annex Chapter 2), however, may be low[173]. Immunization using vaccines based on canine RABV is nevertheless recommended in researchers working on bats, bat lyssaviruses or people visiting bat caves[11,139].

2. Rabies immunoglobulin

The first rabies immune sera were developed by Babes in 1891 and the method further refined by Semple, Fermi, Fuenzalida, Habel or Hosty and others to finally produce specific human antirabies (gamma) immunoglobulin (RIG)[38,86,126,174–180]. Rabies immunoglobulin (RIG) of equine or human origin are neutralizing antibodies targeting the virus's G glycoprotein and are injected in the wound (or in the body or as lavage in case of mucous membrane exposure to bats[181]) to neutralize or reduce the RABV inoculum locally before it can replicate and spread beyond the wound, while the vaccinee produces his or her own antibodies following vaccination. If indicated, rabies immunoglobulin should be injected as soon as possible but can be used within the week after initiating vaccination[182]. There are to date four types of rabies immunoglobulin: (1) RIG of human origin (HRIG) from donors, with a long half-life; (2) RIG of equine origin (ERIG), with a shorter half-life; and (3) highly purified F(ab') fragments produced from ERIG, which have much less unwanted effects than ERIG[61,183,184]. A fourth rabies immunoglobulin based on monoclonal antibodies (Mab) is manufactured and licensed for use in India only. Several Mab RIG are currently being developed.

Despite the high immunogenicity and effectiveness of modern diploid cell vaccines even administered alone[185], the association of RIG with vaccine was shown to be more protective than vaccine alone[186,187]. Importantly, however, much of that work was conducted before the advent of more effective cell-culture or embryonated egg vaccines.

2.1 Using RIG after exposure

RIG is injected in and around the wound and always after lavage. Various guidelines and documents have comprehensively addressed the various technical aspects of RIG administration[181,188–191]. RIG use is described in the next chapter.

2.2 Issues with RIG use

2.2.1 Suppressant effect of RIG on rabies antibody response

Although those titers are considered only a proxy measure of protection[137], administration of RIG with NTV and subsequently with HDCV has led in small experiments to lower percentages of subjects having attained levels considered protective (0.5 UI/mL) compared to vaccination alone[176,192–194]. The same was shown with duck-embryo rabies vaccine in early studies[195,196]. Consequently, a maximum RIG dose was defined to reduce the risk of rabies vaccine response attenuation due to the observed serum-vaccine interference[97,192,197,198]. The suppressant effect of RIG on immune response is countered by booster doses[199–203].

2.2.2 Cost and availability of RIG

The vast majority of people needing RIG do not have access to it, for logistic or financial reasons[71]. Although ERIG are the most widely used, even these are produced in grossly insufficient quantities throughout the World[180,204]. An estimated 0.7 M ERIG doses and 0.3 HRIG doses are manufactured each year outside of China, for an estimated 30 M PEP recipients worldwide annually[18,97]. Producing RIG from animals or humans is technically demanding and biosafety regulations are stringent in most countries. Consequently, rabies immunoglobulin is rare and costly, at 25-35 USD for ERIG and over 250 USD for HRIG, many times the monthly income of most people at risk of rabies in the endemic rural areas of developing countries. Some historical manufacturers are withdrawing from RIG production, which will only make matters worse.

3. Preventing rabies in humans

"Two important facts should always be recollected; viz. the disease may often be prevented; it can hardly ever be cured. Experience has fully proved that when hydrophobia once begins, it generally pursues its dreadful course to a fatal termination, the records of medicine furnishing very few unequivocal and well-authenticated cases to the contrary".

Samuel Cooper, 1823 [205]

As shown in now-developed countries, canine rabies elimination – and consequent, enduring prevention of rabies deaths in humans - can only be durably obtained by large-scale immunization of dogs. This may be achieved in Asia and Africa in the long term. The burden of rabies in Cambodia is presented in Annex Chapter 6. Successful prevention of human rabies cases in the short term, however, relies principally on primary and secondary prevention through rabies vaccination of humans: Rabies

vaccine is therefore administered to present the immune system with glycoprotein epitopes, triggering the rapid production of neutralizing antibodies before the virus can replicate and reach the SNC[206].

3.1 Primary prevention – avoiding exposure and pre-exposure vaccination

Primary prevention of human rabies cases rests upon the prevention of bites and pre-exposure immunization.

3.1.1 Avoiding exposure: preventing animal bites

This is the foundation of rabies control and the original – and to date perhaps the only successful – illustration of the much-vaunted One-Health paradigm.

Avoiding dog bites avoids potential rabies exposure, medical and physical consequences of bites and healthcare expenses. Dog bite incidence has even been reduced in some high-endemicity settings by immunizing dogs against rabies[207,208], a measure that has been shown in several settings to be more cost-effective in preventing human rabies than administration of post-exposure prophylaxis in humans[209,210]. Other crucial measures such as responsible dog ownership, stray dog population control and immunization have long been addressed[75].

Well-cited guidelines for the individual prevention of dog bites recommend avoiding unknown or parenting dogs, remaining "still like a tree" or "lying like a log" should a dog become aggressive[77]. Some education projects in rural communities appear to have been associated in time and space with a reduction in dog bites[211]. Although these recommendations may be effective in reducing the global burden of dog bites and unnecessary PEP in developed countries, their effectiveness on the prevention of bites from rabid animals – especially those with furious rabies – in endemic settings has to our knowledge not been shown.

To our knowledge, there are no internationally (WHO, FAO or OIE)-endorsed guidelines for bite prevention and for responsible dog ownership. OIE briefly mentions responsible dog ownership but does not detail measures to guide individual owners[212]. Few guidelines also are available for policymakers aiming to quantify and undertake monitoring or surveillance and control of dog bites at the community level[213].

3.1.2 Rabies vaccination before a bite (pre-exposure vaccination: PrEV)

Specific primary prevention of rabies in humans is based on pre-exposure immunization against rabies. The rabies vaccine was used for pre-exposure prevention as early as 1885[214]. This is recommended in animal health professionals and in travelers to endemic areas.

Although mass PrEV of the general population in enzootic settings is not recommended by WHO at this time, it may be beneficial in isolated populations[11,215]. The cost-effectiveness of pre-exposure prevention (*vs.* post-exposure) varies according to the setting, and may be favorable only in a setting where the incidence of dog bites is high[216].

At least one case has been described of a rabies death in a person who received pre-exposure (intradermal) rabies vaccine (PrEV)[150,151,158]. This, however, seems to have followed absence of post-exposure boosting and non-response to pre-exposure HDCV vaccine (perhaps due to concurrent chloroquine treatment) rather than vaccine failure despite successful immunization; Experts reassert that no rabies deaths are described in persons who were successfully immunized after PrEV, especially if the recommended post-exposure boosters are administered[61,132].

Wieten *et al.* reviewed the effectiveness of various abbreviated PrEV protocols of single or double 0.1 mL doses, showing effectiveness especially after boosting[217]. In an Australian study, single-dose PrEV administered ID produced a measureable antibody response in 98.2% of travelers by a median of 23 days, while another in New Zealand achieved seroconversion in 95.1% of vaccinees[218,219]. A one-day, four-dose intradermal booster regimen has been used successfully in over 5,000 patients in Thailand[90].

3.2 Secondary prevention after a bite – rabies postexposure prophylaxis (PEP)

Approximately 30 million PEP regimens are administered worldwide each year[18] and hundreds of thousands if not millions of lives have been saved by rabies PEP since it was first successfully administered to Joseph Meister in Paris on July 6, 1885[172]. After washing and antisepsis, rabies PEP is about winning a race to the death with the rabies virus, developing vaccine-mediated antibodies in patients before the virus can replicate sufficiently at the site of inoculation to then pass into neurons where it is protected by the blood-brain barrier and ascends to the CNS (see pathophysiology in Annex Chapter 3). The recommended protocol for rabies PEP includes local as well as general interventions: People in endemic areas should be aware of initial measures following a bite and that they need to seek urgent care if bitten. All health care staff should also be properly trained as to what constitutes timely and effective rabies post-exposure prophylaxis[71,76].

3.2.1 "Beware of Greeks bearing gifts": Timely intervention at the inoculation site

Despite their cunning and fighting skills, would an estimated thirty to forty[220] Greek *commandos* hidden in Ulysse's wooden Horse have overtaken the defenses of the impregnable City of Troy if their numbers had been smaller or quickly reduced by Trojan watchmen?

PEP strategies to prevent infection by infectious agents other than RABV are based on reducing viral inoculum and host vulnerability through antimicrobials (occupational, sexual or perinatal HIV exposure, perinatal HBV, Herpes B, meningitis…)[221–228]. As no proven effective antivirals are available to date for rabies PEP, the aim is to limit the risk of viral replication and diffusion by reducing the viral inoculum through mechanical, chemical (antisepsis) and immunological interventions while the host's immune defenses develop to destroy the virus within days. Fortunately, humans are considered not highly susceptible to RABV[229]. The risk of rabies in unvaccinated persons has long been known to depend on the anatomical site of the infection but also on the importance of the RABV inoculum[2,38,39] (number and severity of wounds, interposition of cloth…) and time of contact (and perhaps the RABV variant).

3.2.2 First aid and wound management

The rabies virus has a lipid membrane and is therefore sensitive to detergents[206]. Immediate and extensive cleansing of the wound or scratch is of paramount importance, using running water and soap or detergent (for at least 15 minutes according to some sources)[61,191] and swabbing[230–232]. After the wound has been thoroughly washed and dried, an antiseptic – preferably povidone-iodine[233] (not opened or past expiration date) - must be applied for five minutes, taking into account that most antiseptics are partially inactivated in the presence of blood[234]. Suturing a bite wound is contraindicated[11,61] but was required in 7.6% of patients in one series of patients managed after a bite by a confirmed or potentially rabid dog[235]. Immediate suture is unavoidable when for example labial occlusion or hygiene of bites below the perineum are compromised. In such cases, suture following debridement must be minimal and be undertaken only several hours after rabies immunoglobulin (RIG) has been injected in and around the wound. Delayed suturing is preferable as it will avoid diffusing RABV in the tissue, also allowing for the decongestion of damaged tissue and improved aesthetic or functional outcome after surgical reconstruction[95]. This multidisciplinary, delayed management was implemented by a consulting surgeon in Pasteur's very first rabies prevention clinic in Paris in 1886[3].

3.2.3 Post-exposure prophylaxis in non-immunized patients

3.2.3.1 Indications for PEP

The fate of the patient is considered to be determined within the first week or so after the exposure to rabies. After the inoculum has been reduced through mechanical action/detersion, antisepsis and in some cases direct action of immunoglobulin (see below), the host's immune defenses must be triggered through PEP to thwart the replication of the virus before it can enter into the nervous system. Indications for PEP (vaccine with or without RIG) vary according to type of exposure and are presented in Table 1.

Table 1: Categories of contact and recommended rabies PEP (Adapted from WHO[11,61])

Categories of contact with suspect rabid animal	Post-exposure prophylaxis measures
Category I – touching or feeding animals, licks on intact skin	None
Category II – nibbling of uncovered skin, minor scratches or abrasions without bleeding	Local treatment of the wound; Immediate vaccination; No RIG unless immune deficient.
Category III – single or multiple transdermal bites or scratches, licks on broken skin; contamination of mucous membrane with saliva from licks, any contact with bats.	Local treatment of the wound; Immediate vaccination and administration of RIG up to Day 7 following the first dose of vaccine*;

*RIG will be replaced by the nascent immune response after that time.

These general WHO guidelines, however, must be interpreted and adapted depending on the type or site of exposure, the nature of the biting animal and/or the bite victim's immune status. Category III management of Category II exposures is indicated if the biting animal is a known reservoir or vector species (bats) or if the patient is known to be severely immunocompromised. Other elements routinely considered at IPC are whether the dog is confirmed rabies-positive. appears sick or the bite was unprovoked, the number of people the animal has bitten, which is higher in rabies-confirmed dogs than in rabies-negative dogs (B. Mollo *et al.*, IPC, ongoing).

When PEP is indicated (with or without RIG), health care workers may choose among several PEP vaccine protocols (Table 2).

3.2.3.2 Intramuscular PEP vaccine protocols

The tedious Pasteur then Semple protocols were ultimately replaced by the Essen and Zagreb protocols. The Essen schedule[78] endorsed by WHO since 1992 now includes the administration of five intramuscular (IM) doses on days 0, 3, 7, 14 and 28. The Zagreb schedule[79–83] is a shorter, four-dose / three session IM protocol endorsed by WHO since 1992 which warrants higher compliance and lower costs for equivalent or higher clinical effectiveness[79–82,236–238]. A four-dose Essen schedule omitting the dose on D28 has been endorsed for use by WHO since 2009[238].

Vaccine doses should be injected IM in the deltoid area in children and adults or the anterolateral aspect of the thigh in babies, not in the gluteal muscle or other fatty tissue as this generates less immunogenicity[95]. Any dose administered in the gluteal site must not be considered as immunizing and should be readministered.

These IM protocols, however, are dose-intensive, consuming a full 0.5-1 mL vial of rabies vaccine for each vaccine session. This led to the development of intradermal (ID) rabies vaccine regimens.

3.2.3.3 Intradermal (ID) rabies vaccination

Pasteur termed his process "vaccination" in honor of Edward Jenner's use of vaccinia[239], a European adaptation of the Chinese then Ottoman process of "engrafting" smallpox scabs into the dermis to

immunize against that disease[240–245]. Inoculation of antigens into the dermis offers the advantage of immediate contact with mast cells, dendritic cells, dermal dendritic cells and Langerhans cells in the presence of extravasation and leucocyctes[246,247], triggering a specific cell-mediated response[247,248]. The high immunogenicity of ID vaccination by direct presentation to these antigen-presenting cells was an important weapon in the fight against smallpox[249], tuberculosis[250] and is being explored for other vaccines[251]: It has been shown to be effective in vaccination against Yellow Fever[252–256], poliovirus[257,258] or seasonal influenza virus[259,260]. Its effectiveness in immunization against HBV has been studied, albeit with conflicting results[261–264].

A comprehensive overview of the development of intradermal rabies vaccination was published by Warrell[206]. First tested in the early 1960s[265], an intradermal regimen was operationally developed in the early 1980s[248,266–271]. This strategy was further refined using Vero-cell derived vaccines by the expert rabies team at Bangkok's Queen Saovabha – Thai Red Cross (TRC) center[95,272]. It was endorsed by the WHO expert committee since September 1991[128] and the WHO Strategic Advisory Group of Experts on immunization (SAGE) since 2000-2002[273,274]. To September 2017, the TRC regimen associates four sessions of two ID injections of 0.1 mL, whether the vaccine is purified chick embryo- or purified Vero cell derived[11,206,217,275–282]. In a 2002-2003 study in the Philippines, Quiambao *et al.* [282] assessed the effectiveness of TRC intradermal PEP in 113 subjects with Category III bites by confirmed rabid animals (97% by dogs and 3% by cats) who also received RIG, demonstrating serological and clinical equivalence of an ID and an IM schedule[235].

Doses of reconstituted rabies vaccine are drawn into syringes, preferably insulin syringes to accurately measure the small dose delivered, being careful to remove dead space[188]. After local disinfection and using intradermal needles, one dose of 0.1 mL is injected in each of the two deltoid areas, creating a papule ("peau d'orange") caused by an intradermal collection as for intradermal testing for tuberculosis. This procedure causes some pain and requires speed and skill, especially in squirming children. Furthermore, administering several doses as in several proposed abbreviated regimens is time- and labor-intensive, especially in busy rabies clinics.

Despite the smaller antigenic inoculum, differences in the chemokine/cytokine activation pathways may offer an explanation for the now firmly established clinical equivalence of rabies vaccination through IM and ID routes[246] (see below). The Thai Red Cross ID regimen counts among WHO-recommended protocols to September 2017[11,80,85,283]. Table 2 shows the regimens in use to September 2017 at Institut Pasteur du Cambodge where the vast majority of patients receive intradermal PEP. Table 3 shows the various WHO-endorsed and experimental regimen.

Table 2: Antirabies vaccination protocols at Institut Pasteur du Cambodge, Cambodia, 2015.

	Day	D_0	D_3	D_7	D_{21}	D_{28}
PRE-exposure	Dose (0.5mL) IM	1		1		1 or 1
	Dose (0.1 mL) ID	1		1		1 or 1
POST-exposure*	Dose (0.5mL) IM	2		1		1
+ RIG if Category III	Dose (0.1 mL) ID	2	2	2		2
or even in Category II/III	Immune deficient IM	1	1	1		1
in case of immune deficiency	Previously immunized IM	1	1			
NO RIG in pre-exposure immunized persons	Previously immunized ID	2	2			

*see WHO Categories of exposure[11,61]

Table 3: Various pre-exposure and post-exposure regimen, WHO-approved or otherwise.

		D_0	D_3	D_7	D_{14}	D_{21}	D_{28}	D_{90}	Remark
Pre-exposure prophylaxis									
WHO-approved 2010									
Intramuscular (IM)	IM	1		1		1 or 1			11
Intradermal (ID)	ID	1		1		1 or 1			11,150,284
Experimental									
Soentjens P *et al.*	**ID**	**1**		**1**					285 **WHO-endorsed in 2018**[113]
Wongsaroj P *et al.*	ID	1				1			286
Lau C *et al.*	ID	1							98.2% response[218]
Shaw MM *et al.*	ID	1							95.1% response[219]
Post-exposure prophylaxis									
WHO-approved in 2010									
TRC until 2007	ID	2	2	2			1	1	273,274
TRC since 2007	ID	2	2	2			2		287
Zagreb	IM	2		1		1			~100% response by D14[79,288]
Essen	IM	1	1	1	1		1		~100% response by D14[79,288]
4-dose/2 weeks	IM	1	1	1	1				84
Experimental									
IPC	**ID**	**2**	**2**	**2**					289**; WHO-endorsed in 2018**[113]
Narayana, Sudarshan, Shantavasinkul *et al.*	ID	4	4	4					90,92,94
Liu H *et al.*	IM	2	1	1					288
Huang G *et al.*	IM	2		1					93
Warrell MJ *et al.*	ID	4		2		1			89; Endorsed 2007[287]; Retrieved 2010[11]
Khawplod P *et al.*	ID	8		4		1	1		87
	ID	8		4					
	ID	4	4	4					
Boosters in immunized									
WHO-approved in 2010									
TRC	ID	2	2						11
Experimental									
Tantawichien T *et al.*	ID	4							290
Sirikwin S *et al.*	ID	8	8	8	8		8		291
Khawplod P *et al.*	ID	2	2	2					292
	ID	4	4	4					
	ID	8		4					

3.2.4 Estimations of overall rabies PEP effectiveness

Although rabies PEP is widely considered as one of the most successful and cost-effective health interventions in endemic settings, very few studies have provided data on rabies PEP effectiveness in real-world situations[293]. Most available data on protection by rabies PEP originate from immunological studies in healthy volunteers, which fall short of the degree of evidence needed to evaluate capacity of new PEP regimens to spare lives in the field.

3.2.4.1 Estimates of rabies transmission in the absence of PEP

Several studies provide data on survival in patients who received PEP after a bite by a confirmed rabid or suspected rabid dog[183,294]. Based on 19thC data, the overall risk of transmission after the bite of a rabid dog has been estimated approximately at 20% in absence of PEP[38,40].

3.2.4.2 Estimates of PEP effectiveness

Available data on PEP effectiveness and failures may be seriously biased. These were obtained mainly through passive surveillance of human cases bitten by dogs which overwhelmingly were not tested for rabies. Furthermore, survival may have improved also because of greater awareness, easier access and systematic PEP after a dog bite and not only after a bite from a confirmed or suspected rabid dog[295]. Various available data are shown in (Table 4).

Canine-mediated human rabies is a near-constantly fatal disease (see Annex Chapter 4 for clinical description and outcome). Understandably, no field vaccine studies have data using placebo or an exposed but unvaccinated control group, except for four "natural experiment" studies, some very dated, which examine outcome in persons unable or unwilling to receive PEP. A publication from 1923 provided outcome in persons who received PEP using Semple vaccine (2.9% deaths among 812 persons), comparing it to outcome in persons coexposed to the same presumably (but untested) rabid dog but who did not refer for PEP (6.2% of 1,362 persons)[123]. The authors conclude that PEP saved one out of two persons who would have died of rabies after being bitten by a suspect but untested dog. Another publication mentions a 1946-1956 series of 541 persons bitten by confirmed rabid dogs in India, with 8.8% rabies deaths among the 315 who received PEP with Semple vaccine compared to 48% fatalities among 106 who did not[265].

These success rates have profoundly changed with the advent of modern vaccines. In a controversial 1984-1985 study in Thailand, nine (45%) of 20 persons who referred for deep wounds inflicted by confirmed rabid dogs and who refused NTV contracted rabies and died, demonstrating 100% effectiveness of PEP in the field[296]. In a study by Quiambao *et al.*, 57 persons with confirmed exposure to RABV who received intramuscular Vero-cell vaccine were all alive at one year[235]. In another study by Quiambao *et al.*, 110 patients aged 5-60 years who received intramuscular PEP and equine RIG after

being bitten by confirmed rabid dogs were all alive at one year[282]. Kalmotham *et al.* found 100% survival at one year among 131 patients who received various PEP regimens after Category II/III exposure to confirmed RABV[297]. In a study investigating the efficacy of PEP boosters, Shantavasinkul *et al.* documented survival in 253 participants who had confirmed rabies exposure and received PEP at least one year earlier[298]; Although it mentions that there were no reported deaths, this study provides no follow-up data on patients who received PEP but may not have survived.

Table 4: Summary of various clinical outcome studies in persons bitten by rabid or suspected rabid canines which with or without rabies post-exposure prophylaxis.

	Vaccine used	PEP*	Died (CFR; 95%CI)	No PEP	Died (CFR; 95%CI)	Vaccine effectiveness	
						Computed among followed-up**	Estimated by comparing to 20%-40% theoretical transmission data
	Confirmed rabid dogs						
Cornwall JW *et al.*, India, 1923[123]	Semple NTV, IM	-	-	423	148 (35.0%; 30.4-39.7%)	NA	NA
Pasteur Institute of India, 1946-1968 [299]	Semple NTV, IM	844	62 (7.35%; 5.6-9.1%)	215	122 (48.1%; 49.8-63.5%)	87.05%	-
Thongcharoen[300] *et al.* Thailand, 1980s	IV Vero-cell	27	0 (0.0%; 0.0-0.2%)	-	-	-	100.00%
Sitthi-Amorn *et al.*, Thailand, 1984 [296]	Semple and other	661	0 (0.0%; 0.0-0.01%)	20	9 (45.0%; 23.2-66.8%)	100.00%	-
Quiambao *et al.*, Philippines 1994-1995 [235]	IM Vero cell + ERIG	57	0 (0.0%; 0.0-0.1)	-	-	-	100.00%
Kamoltham *et al.*, Thailand 1996-2001[297]	IM or ID cell-based + ERIG	188	0 (0.0%; 0.0-0.3%)	-	-	-	100.00%
12 Rabipur® studies cited in Giese *et al.* [301] (excl. [282])	IM or ID, ± RIG, rabid dogs	646	0 (0.0% - 0.0 - 0.008%)	-	-	-	100.00%
8 Verorab® studies cited in Toovey[80] (excl. [302])	IM, ID or SC, ± RIG, all rabid dogs	360	0 (0.0% - 0.0 - 0.01%)	-	-	-	100.00%
Jaiiaroensup *et al.*, Thailand 1994-1996[302]	IM or ID cell-based + ERIG, all rabid dogs	84	0 (0.0%; 0.0-0.6)	-	-	-	100.00%
Quiambao *et al.*, Philippines 2002-2003 [282]	ID PCECV + ERIG, rabid dogs	110	0 (0.0%; 0.0-0.5%)	-	-	-	100.00%
Quiambao *et al.*, Philippines 2003-2004 [183]	IM or ID Vero cell + ERIG, dogs, cats	146	2 (1.4%; 0.2-4.9%)	-	-	-	100.00%
Bharti *et al.*, India, 2014 [303]	ID Vero cell ± ERIG	26	0 (0.0%; 0.0-0.2%)	-	-	-	100.00%
Hampson, Tanzania, 2002-2006 (personal comm.)	IM cell-based vaccines	15	0 (0.0%; 0.0-0.3%)	-	-	-	100.00%
Tarantola *et al.*, Cambodia, 2003-2014[289]	ID cell-based + local ERIG, all rabid dogs	1,739	3 (0.17%; 0.03-0.50%)	-	-	-	99.14% - 99.57%
Tarantola *et al.*, Cambodia, 2003-2014[289]	ID cell-based + local ERIG, untested dogs	1,093	0 (0.0%; 0.0-0.005%)	-	-	-	100.00%
Salahuddin *et al.*, Pakistan, 2009-2014[304]	ID cell-based + local ERIG, untested dogs	2,916	2 (0.1%; 0.0 – 0.3%)	-	-	-	99.66% - 99.83%
Total	(vaccines, routes and exposures too varied)	NA	NA	658	279 (42.4%; 38.6-46.2%)	NA	NA

* In some cases, incomplete; ** Computed as VE = 1 – RR where RR is the relative risk of developing rabies in persons without PEP compared to those who received PEP [305]; NA: Not applicable.

Data on PEP effectiveness after a bite by a confirmed rabid animal will vary greatly in terms of healthcare seeking habits and ease of access to timely and adequate PEP. Case-fatality rates rise if patients refer only for very severe wounds to high-risk anatomical sites, if they seek PEP belatedly, if the vaccines are not immunogenic at the production site or at point-of-care (cold chain issues) or if patients have no access to RIG. As in a series from Pakistan with 40% rabies deaths despite full-course PEP using NTV, the discrepancy between efficacy estimated by immunological response in controlled laboratory conditions and real-world effectiveness can be due to many factors such as delayed referral, protocol deviation and cold chain to preserve vaccine effectiveness[306]. The same authors mention that, in the 1950s, Indian-produced Semple type vaccine was estimated to have an effectiveness of 84% in patients who received a full course of rabies PEP, which dropped to 58% in those who received an incomplete PEP regimen[306].

The efficacy of modern rabies vaccines has therefore been found to be 100% in controlled studies, but its effectiveness after bites by confirmed rabid dogs seems somewhat lower in real-world situations, as shown by our field experience (Tarantola A. *et al*., submitted).

3.2.5 PEP effectiveness: Intradermal *vs.* intramuscular

In a study in Thailand between 1996 and 2001, Kamoltham *et al.*[297] reviewed 188 patients exposed to biologically-confirmed rabies, 20 (10.6%) of whom had received the intramuscular Essen regimen and 168 (89.4%) who received intradermal PEP (ID TRC). Among these, 75 (39.9%) had Category III exposure, including six (8%) who received IM Essen and 69 (92%) received ID TRC. All 188 patients were alive at one year after exposure (Table 4).

A similar clinical study by Jaiiaroensup *et al.* concluded to 100% survival at 3 years in rabies-exposed patients who received IM or ID PEP[302]. Another study by Bharti *et al.* on a more limited sample of 26 patients bitten by rabid dogs and who received ID post-exposure rabies vaccine and RIG only in the wound found 100% survival at one year or more[303]. Several studies on the dynamics of rabies antibodies following one or the other administration route concluded to a proxy antibody response equivalent to clinical protection[248,307–309]. Detailed comparisons by vaccine and protocol have been comprehensively reviewed by Toovey[80] or Rupprecht *et al.*[310]

It is estimated that 60-80% of rabies vaccine used worldwide is now administered intradermally. Although ID vaccination would theoretically reduce costs by 60-80%, one large-scale field implementation in Thailand documented real cost savings as closer to 40%[297] and another in the Philippines at 25%[273].

3.2.6 Rabies PEP failures

Rabies PEP failures were described as early as the vaccine itself was introduced. Around one year after the first PEP, L. Pasteur in August 1886 reported 3 (0.2%) deaths (whether the case of Louise Pelletier is included among these deaths is unclear) among 1,235 PEP recipients after the bite of a potentially rabid animal[3], while another source speaks of 21 (1.0%) deaths among 1,986 recipients (including one from Bombay, India) by 22 August of that same year[311]. In 1887, Vulpian documented 12 (0.7%) deaths among 1,726 PEP recipients, for an expected number of approximately 264 (15.3%) rabies deaths if PEP had not been administered[312].

A total of 47 (0.1%) fatal outcomes were considered PEP failures among the 52,751 persons who received NTV PEP between 1891 and 1953 at the Institut Pasteur in Saigon[313]. The proportion of those truly exposed to rabies, however, is undocumented. From 1963 to 1968, for example, approximately 30,000 persons received PEP in the USA after confirmed or potential exposure to rabies, among which 5 (0.02%) died of rabies[314]. Post-exposure prophylaxis failures became less frequent as vaccines improved.

Failures of rabies PEP using modern vaccines are now exceedingly rare[61,84,315–327]. Between 1992 and 1999, more than 600,000 PEP were administered in Thailand, a rabies-endemic country, with 13 rabies (0.002%) deaths documented through passive surveillance[42]. As stated in the literature, these PEP failures generally occurred due to long delays before referral or deviations from the WHO-recommended protocol such as suturing before PEP[11,84,132,183,325,328,329] (often imposed by operational or anatomical constraints such as transfixing wounds to the cheek or the perineum), omitting to infiltrate all the wounds with RIG[61,95] or presumed direct RABV inoculation into a nerve.

3.2.7 Comparing and validating PEP protocols and vaccines

Taken altogether, clinical experience and new epidemiological data show that ID rabies PEP is comparable to IM in saving the lives of people bitten by rabid dogs[330–335]. But if PEP is to be improved and made more cost-effective, how can regimen and vaccines be reliably compared?

Although serological studies show that some regimen produce higher antibody titers than others, antibody titers rising at least once above 0.5 IU/mL are considered a reliable proxy for protection (including legally when considering boosters in occupationally-exposed personnel). The clinical outcome in rabies-exposed persons is determined by: 1/ the mechanical reduction of the inoculum; 2/ Rabies immunoglobulin use (to be better studied with modern vaccines); and 3/ the immune response within the first few days following exposure. Higher and more persistent titers might in the long term provide protection against subsequent exposures but long-term persistence of high antibody titers after PEP does not impact the outcome of the exposure for which that PEP is administered[336]. There is definite immune response overshoot and "virus overkill": long-term anamnestic response is also considered to

be protective and expert clinicians consider that no deaths occur in people that have been immunized once in their life.

In this author's opinion, comparison of regimens' effectiveness and validation of new protocols must therefore not rely on the peak and duration of immune response in healthy volunteers. It must rely instead on 1/ The percentage of persons who have antibody titers ≥0.5 IU/mL at a given time point; 2/ Those capable of mounting an anamnestic response and - perhaps most importantly - 3/ Clinical outcome data in people who did and who did not receive RIG with vaccine after category II or III exposure to confirmed rabid dogs. Clinical studies in healthy volunteers may suffice only to validate one vaccine's noninferiority compared to a validated vaccine for a given PEP schedule.

3.2.8 Operational constraints linked to intradermal vaccination

The Rabies Prevention Center at Institut Pasteur du Cambodge (rpc@ipc) opened at the same time as IPC on 27 March 1995. Dr. Jean-Louis Soarès is to be credited with introducing intradermal PEP with nerve-tissue vaccine (Fuenzalida) from the onset, with 8,607 PEP referrals in 1996, 76% of which were Phnom Penh residents. From 2000-2014 incl., a total of 252,772 patients received intradermal rabies PEP at IPC, including 203,519 using a Vero-cell vaccine (used exclusively after 2003) in unvaccinated patients after a bite by a potentially rabid dog, 49% of which were from Phnom Penh.

Constraints linked with ID PEP use at IPC's rabies prevention clinic include the following: (1) ID use is only possible in centers with sufficient turnover for ampoules to be used within six hours and not having to discard them before they are empty; (2) The absolute number of injections per patient (two per session) becomes an issue in very busy centers like that of IPC which provides care to over 21,000 patients per year; (3) Time and care needed to reconstitute vaccine using sterile powder and diluent and then to precisely fraction two 0.1 mL doses per syringe, one for each deltoid area; (4) Skill required to inject one 0.1 mL dose quickly but accurately in the dermis, twice over for each patient, including young (squirming) children. This has also been documented in the Philippines[273]. Importantly, the immune response to would-be low-volume intradermal doses accidentally injected in subcutaneous tissues has been reported to be the same as intradermal vaccination, at least for purified Vero-cell rabies vaccine[337].

4. Abridged rabies prevention protocols in humans

Early on, clinicians and expert committees have sought to maximize PEP effectiveness and reduce risks, recommending and conducting research on the efficacy of reduced vaccine schedules[84,338].

4.1 Historic protocols

Pasteur's first post-exposure prophylaxis (PEP) using Pasteur's attenuated vaccine required daily injections over twelve successive days[2,38]. Later protocols ranged in duration from 15-21 days in Paris, up to 40 days for severe exposures in some centers, while other centers attempted to shorten the protocol duration to ten days[38]. Semple's own phenol-inactivated nerve-tissue vaccine being less immunogenic, PEP required up to 29 doses. The better tolerated suckling mice rabies vaccine regimen developed by Fuenzalida and Palacios in Chile also required 10-15 doses[10,328], later reduced to 8 doses[339] with at least one attempt to reduce its posology to four doses in human volunteers[340,341]. These attenuated or inactivated NTV – containing high titers of myelin and other animal proteins - required numerous doses and injections, caused significant pain (based on personal experience, in 1972…) and adverse effects. Even the first human culture vaccine used in PEP, Vnukovo-32, required up to 25 injections[78]. As newer, better tolerated and more antigenic human diploid-cell-based vaccines were introduced, protocols – initially only intramuscular and later intradermal - could be and indeed were abridged[84,85,185,342].

4.2 Abridged pre-exposure rabies vaccination (PrEV)

4.2.1 Abridged intramuscular pre-exposure rabies vaccination

As per WHO recommendations, pre-exposure regimen now associate intramuscular (IM) injection of 0.5 mL or 1 mL doses of vaccines (depending on the producer's indication) during each of three sessions (on Days 0, 7, and 21 or 28)[11].

4.2.2 Abridged intradermal pre-exposure rabies vaccination

Intradermal (ID) injections of rabies vaccines have been shown to be noninferior to IM administration[248,307,308]. A one-day ID regimen using various numbers of HDCV vaccine doses was found ineffective[343] but several accelerated pre-exposure regimens have demonstrated effectiveness[217]. In Thailand, a study by Shantavasinkul *et al.*[90] in 131 healthy Subjects showed that a four-site, one-week pre-exposure ID regimen produced adequate levels of neutralizing antibodies in all subjects, albeit for a lower time period in RIG recipients. Another Thai study found that a one-week 2-2-2 ID regimen produced lower response at D7 but equivalent response at D14 compared with a one-week 4-4-4 ID regimen in a small number of volunteers[87]. As of September 2017, intradermal pre-exposure administration is endorsed by WHO for one injection of 0.1 mL only (single dose) during each of three sessions (on days 0, 7, and 21 or 28)[11]. A study on a one-week pre-exposure regimen using two single 0.1 mL ID doses at Day 0 and Day 7 in 420 travelers concluded to 94.5% effectiveness[344]. Another study in 55 healthy volunteers in Thailand using one 0.1 mL ID doses at Days 0 and 21 had 100% effectiveness at Day 35[286]. A preliminary study of one-visit, two-dose pre-exposure ID vaccination followed at one

year by one-session, 4-site ID boosters showed promising results in healthy volunteers[345]. Recently published data from Soentjens *et al.* in Belgium yielded encouraging results[285]; At the time of writing PrEP is likely to be shortened to two sessions (on Days 0 and 7) in immunocompetent persons in 2018 recommendations.

4.3 Abridged rabies post-exposure prophylaxis (PEP)

4.3.1 Abridged intramuscular PEP

A 3-months protocol termed the "Essen regimen" associating IM injections at Days 0, 3, 7, 14, 30 and a 90 booster was "temporarily recommended" at a 1975 WHO conference in Tehran[78]. This "Essen" IM schedule was subsequently shortened to 5 doses[346] and remains a reference regimen to date. The "Zagreb" 3-week IM schedule (four-dose, three-session protocol on Days 0, 7 and 21) was developed by the Zagreb Public Health Institute in the 1980s and demonstrated noninferiority[79–83]. A 2-week, shortened IM protocol has also been developed for immunocompetent subjects also receiving RIG[84]. All three protocols are endorsed by WHO[11]. A failed attempt at a one-week IM regimen (3 doses at Day 0 and 1 dose at Day 7) initially produced encouraging results, with a humoral response after Day 5 which, however, was suppressed for two weeks by concomitant RIG[128].

Intramuscular PEP protocols were further shortened due to a vaccine shortage which lasted from mid-2007 to early 2009. Thank to this interruption in supply, rabies and vaccine experts at the US Centers for Disease Control and prevention were able to recommend abandoning the fifth dose of the Essen regimen[84,132,310], although this protocol is recommended for use in healthy, immunocompetent persons only. In a recent study in healthy volunteers, Huang *et al.* showed that one-week IM PEP produced immunity considered protective in 100% of subjects by Day 14[93].

4.3.2 Abridged intradermal PEP

The only intradermal protocol approved by WHO to September 2017 has been developed at Thai Red Cross (TRC) in Bangkok, Thailand. This highly effective and cost-sparing protocol associates injection of one intradermal 0.1 mL dose in two sites on each of three sessions at Days 0, 3, 7 followed by a single 0.1 mL dose at Days 30 and 90[273]. This was simplified and shortened in WHO recommendations since 2005[11,92]: The updated TRC post-exposure regimen in unvaccinated subjects now associates intradermal injections 0.1 mL in two sites on days 0, 3, 7 and 28 (no session at D90)[11,85].

Studies by Thraenhart *et al.*[347] showed that doubling initial doses led to higher antibody response, although this response does not occur earlier[92]. A study on shortened one-week intradermal PEP in healthy volunteers using four ID doses on each of three sessions found 100% effectiveness as evidenced by neutralizing antibody titers[92].

Over the past few years, several studies on small numbers of healthy volunteers have been published by Thai or Indian teams showing the effectiveness of three sessions of four intradermal injections[90,348]. One of these studies in India shows that the immune response at D14 after a one-week protocol of three consecutive injections at Day 0, Day 3 and Day 7 of four ID doses each is comparable to a 5-session regimen in healthy volunteers[348]. At 12 intradermal doses, the workload of this one-week, three session protocol, however, remains much too high.

As did Beran *et al.* in healthy volunteers[349], Ravish *et al.* showed that all dog-exposed recipients of a purified chicken embryo vaccine administered intradermally presented by Day 14 antibody titers considered protective[350].

5. Promoting timely and affordable access to adequate PEP in developing countries

Efforts have thus been made for decades to shorten and simplify PEP protocols[172]. This is a public health priority in the developing setting, where shortening protocols helps reduce doses, injections and costs linked with vaccines, transportation and being away from work[351,352].

Post-exposure prophylaxis is a cost-effective intervention[310,353,354], especially in rabies-endemic settings. The World Health Organization is spearheading a global effort to eliminate canine-mediated rabies by 2030[99,355], a strategy endorsed by ASEAN countries including Cambodia[356–359]. The ASEAN strategy is based on vaccinating dogs – which will take decades - but also improving access to timely and effective PEP[100], especially in Cambodia where this is accessible essentially in Phnom Penh, the capital.

It is well-established that distance and cost remain major obstacles to accessing timely and adequate PEP for rural populations of developing countries[352]. Returning to the capital for the 4th intradermal session at D28 contributes to incur unaffordable costs to bite victims in Cambodia. How then can access be improved?

In Cambodia the answer may to a large extent lie in:

1. Performing a baseline assessment of available sources of PEP, and current use and obstacles to use of timely and adequate PEP;
2. Improving geographical access to timely and effective PEP by optimally positioning peripheral clinics to promote referral, reduce time and resources spent for referral as well as to reduce PEP completion; and

3. Shortening intradermal PEP protocols to reduce the number of sessions, direct costs of vaccines and indirect costs due to repeated transportation and/or accommodation costs, especially when an adult must accompany a child bitten by a suspected rabid dog.

These crucial actionable issues are the focus of the studies presented herein to qualify for this Doctorate in Philosophy of Science at Paris Diderot (Paris 7) University – Paris Sorbonne University, on "EPIDEMIOLOGY AS A TOOL TO IMPROVE PREVENTION OF HUMAN RABIES: LOCAL AND GLOBAL HEALTH IMPLICATIONS OF POSTEXPOSURE PROPHYLAXIS DATA, INSTITUT PASTEUR DU CAMBODGE, 2003-2014."

Part 2: Assessing the obstacles to timely and effective PEP in Cambodia

6. Rabies Vaccine and Rabies Immunoglobulin in Cambodia: Use and Obstacles to Use

6.1 Background and rationale

The Rabies Prevention Center at Institut Pasteur du Cambodge (rpc@ipc) was created at the same time that IPC was reopened, on 27 March 1995 and was specifically mentioned in the memorandum of understanding signed between the Institut Pasteur International Network and the Cambodian health authorities. The data available for the first completed year were collected in 1996, showing 8,607 rabies post-exposure (PEP) referrals, 76% of which were Phnom Penh residents. In 2013 we sought to re-examine the updated situation and services rendered to the population, identifying avenues for progression, if any.

6.2 Method

This study was two-pronged. First, we sought to update our data on PEP referrals to rpc@ipc. This was done by extracting and analyzing PEP data for the previous completed year (2012). We then sought to document the number of PEP referrals at other sites throughout the country using a web-based survey.

The data was extracted from the overall IPC database. This EpiData (EpiData Association, Odense, Denmark) database serves as a baseline risk assessment and PEP monitoring tool. It is informed as part of clinical routine work by the same, trained clinical team using a standardized questionnaire for each patient. Patient sociodemographic and bite characteristics (number and anatomical sites of wounds…)

are informed, as well as dog status (sick-looking dog, unprovoked bite …) and the nature and timing of the PEP received. These data were imported into and analyzed using Stata 13 (Stata Corp., College Station, TX, USA). We also examined vaccine and rabies immunoglobulin order forms from IPC for 2012.

We contacted the pharmaceutical company which sells and distributes Vero cell-based rabies vaccine in Cambodia. This company had data on the number of doses it sold each year in-country, as well as an estimation of the overall number of all rabies vaccine doses used in Cambodia each year. Furthermore, it listed the in-country institutions which ordered and administered rabies vaccine and immunoglobulin each year. These institutions were contacted by the author using SurveyMonkey®, (SurveyMonkey, Palo Alto, CA, USA) to document their PEP referral data. These were then aggregated and anonymized. Data on vaccine and immunoglobulin costs were collected on the internet.

6.3 Results

A total of 20,610 persons were referred or self-referred to IPC for PEP after exposure to a potentially rabid animal in 2012. These patients originated from 22 of Cambodia's then 23 provinces. In 2012, 98.4% of patients originated from only 10 of these provinces (incl. 41% from Phnom Penh) while these 10 provinces account for 66.8% of the total population of Cambodia. Of the 20,609 patients documented for type of injury, 6 (0.03%) referred after a scratch by an animal, 20 (0.1%) after an animal lick on nonintact skin, 1 after a bite + scratch and 20,582 (99.9%) after an animal bite. Of these, 6,362 (30.9%) were considered deep (Category III according to WHO classification[11]) and 14,220 were considered superficial bites (Category II). Nine percent of the Category III patients received equine rabies immunoglobulin (ERIG).

The vaccine distribution survey identified five PEP centers other than IPC and received an answer from all of these. These five centers - one foundation Hospital in Siem Reap and two Embassy clinics, one governmental institution and one private healthcare Center, all in Phnom Penh - managed a total of less than 1,500 patients in 2012. Based on vaccine distribution data, an additional 10,000 persons were administered pre- and/or post-exposure vaccine doses in 2012 in the country.

Most centers resorted to intramuscular PEP, administering a full 0.5 mL dose during each of four sessions. The cost of PEP was estimated using web-based resources. Based on wholesale pricing, the cost of a full course of intramuscular PEP was estimated on average at US$31.50 compared to US$12.60 for a four-session, one-month dose-sparing Thai Red Cross intradermal regimen. When indicated, the wholesale price for one dose of ERIG ranged between US$20 and US$35, depending on the manufacturer.

6.4 Discussion and conclusion

These data gathered by a combination of strategies (data extraction and analysis, web-based survey) confirmed that IPC was by far the largest PEP center (2/3 of PEP delivered) in Cambodia in 2012 and that it alone resorted to intradermal PEP.

Compared to the very first year with reported IPC data, patient referral in 2012 increased dramatically with a larger proportion of patients originating from rural provinces (outside Phnom Penh). Although referred or self-referred patients originated from all provinces save one, however, populated areas from the west of the country seemed underrepresented. This may have reflected serious challenges in geographical access to timely and adequate PEP for residents of Western provinces.

A Cambodian farmer's monthly income in 2012 was between US$60 and US$80. This makes direct costs (vaccine, ERIG …), indirect costs (repeat transportation to and accommodation in the Capital, especially if the patient is an accompanied child) and loss of income related to PEP unaffordable for the most at-risk populations. Abridging the PEP regimen to three sessions over one week only would help reduce the direct as well as indirect costs for rural residents and improve equity in accessing timely and adequate PEP in Cambodia.

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6.1 Article 1

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BRIEF COMMUNICATION

Rabies Vaccine and Rabies Immunoglobulin in Cambodia: Use and Obstacles to Use

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Background. Authorities have pledged to eliminate canine rabies by 2020 in Cambodia, a country with a very high rabies burden. Logistic and financial access to timely and adequate postexposure prophylaxis (PEP) is essential for preventing rabies in humans.
Methods. We undertook a survey of the few identified sites where PEP rabies vaccination and rabies immunoglobulin (RIG) are available in Cambodia. We examined the Rabies Prevention Center at Institut Pasteur du Cambodge (rpc@ipc) database and rpc@ipc order forms for 2012 to assess vaccine and RIG use. We conducted a rapid internet survey of centers that provide rabies vaccine and RIG in Cambodia, other than rpc@ipc.
Results. The cost of a full course of intramuscular or intradermal PEP in Cambodia, with and without RIG, was also estimated. Rabies vaccination is free of charge in one foundation hospital and is accessible for a fee at Institut Pasteur du Cambodge (IPC), some institutions, and some Cambodian private clinics. In 2012, 27,500 rabies vaccine doses (0.5 mL) and 591 equine RIG doses were used to provide intradermal PEP to 20,610 persons at rpc@ipc following animal bites. Outside of rpc@ipc, an estimated total of 53,400 vaccine doses and 200 RIG doses were used in Cambodia in 2012. The wholesale cost of full rabies PEP was estimated at 50% to 100% of a Cambodian farmer's monthly wage.
Conclusions. Local populations and travelers cannot be sure to locally access adequate and timely PEP due to high costs and low access to RIG. Travelers to high-endemic areas such as Cambodia are strongly encouraged to undergo pre-exposure vaccination or seek expert advice, as per World Health Organization (WHO) recommendations. State-subsidized, pre-positioned stocks of human vaccine and RIG in bite management centers would extend the rabies prevention centers network. Support from Institut Pasteur du Cambodge for staff training, cold chain, and quality control would contribute to reducing the risk of rabies deaths in Cambodia.

With an estimated dog for every two to three inhabitants (Institut Pasteur du Cambodge, unpublished data), the incidence of dog bites is extremely high in Cambodia.[1] Cambodia is also highly enzootic for rabies: an estimated 800 human rabies cases are thought to occur each year in a population of 14 million inhabitants.[2] This translates into an estimated annual incidence of about six rabies deaths per 100,000 inhabitants [5.8/100,000; 95% confidence interval (CI) 2.8–11.5], comparable to the 2006 incidence of road deaths in the European Union.[3]

Cambodia like other countries of the ASEAN + three group has committed to eradicate canine rabies by 2020.[4–7] But Cambodia faces numerous challenges and there are, to our knowledge, no canine vaccination, no validated strategy, no funding, and insufficient expertise dedicated to canine rabies elimination to date. In Cambodia, human rabies cases are avoided solely by postexposure prophylaxis (PEP) available to a minority of patients.[8] Immediate wound management and vaccine PEP[9] are essential in reducing risk, but rabies immunoglobulin (RIG) use is also considered a mainstay of prevention.[10] We undertook this study to identify the gaps in access to timely and affordable PEP in Cambodia and their underlying causes.

Methods

Created in 1995, the Rabies Prevention Center at Institut Pasteur du Cambodge (rpc@ipc) is the largest rabies prevention clinic in Cambodia, providing postexposure coverage for the southeastern provinces and most of

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the Cambodian population. Using Stata 13 (StataCorp, College Station, TX, USA) we examined the rpc@ipc database (EpiData, EpiData association, Odense, Denmark) and rpc@ipc order forms for 2012 to assess vaccine and RIG use. We mapped the province of residence of the referrals to rpc@ipc in 2012 using ArcMap 10.0 (Esri, Redlands, CA, USA) to estimate served areas.

Using SurveyMonkey (SurveyMonkey Inc., Palo Alto, CA, USA),[11] we also conducted a rapid survey of identified centers other than rpc@ipc, which may provide RIG in Cambodia. The data were anonymized and compared with estimated dose usage and associated costs in Cambodia for all vaccines and manufacturers in 2012, the latter kindly provided by Sanofi Pasteur, the main supplier in Cambodia of WHO-prequalified purified inactivated rabies vaccine prepared on VERO cells.[12] There are no other available estimates. These data also pertained to the wholesale costs of immunoglobulin from various manufacturers in Cambodia, which were used to estimate the cost of a full course of intramuscular or intradermal PEP, with and without immunoglobulin.[9]

Results

Rabies vaccination is free of charge in one foundation hospital and is accessible for a fee at IPC, in some institutions, and in Cambodian private clinics (Table 1).

Rabies PEP at rpc@ipc

In September 2010, a partial cost recovery scheme was introduced: a full course of four sessions of two 0.1 mL intradermal doses is charged US$10 at rpc@ipc, with an additional cost of US$35 in case equine RIG (ERIG) is indicated. In some cases, full rabies PEP including ERIG is offered free of charge.

In 2012, a total of 125,100 ID (0.1 mL) rabies vaccine doses from 27,500 doses of 0.5 mL (4.5 doses of 0.1 mL per 0.5 mL vial, due to dead space in the syringes before changing to insulin syringes) were administered to 20,610 persons at rpc@ipc following animal bites. Of the 22 provinces from which dog bite victims were referred to IPC, 10 contributed more than 100 animal bite cases. These provinces—Phnom Penh, Kandal, Kompong Cham, Prey Veng, Takeo, Kompong Speu, Kompong Chhnang, Kampot, Kompong Thom, and Svay Rieng—accounted for 20,292/20,610 (98.4%) of the animal bite cases managed at rpc@ipc in 2012 (Figure 1). The population residing in these 10 provinces constitutes 66.8% of the total population of Cambodia. In all, 591 (9.3%) of the 6,362 patients with Grade III injuries also received ERIG in 2012. Of the 227 persons referred to rpc@ipc after exposure to confirmed rabid animals' bites, 99% received ERIG. Of these 227 cases, 45 (19.8%) originated from Takeo, 39 (17.2%) from Kampong Cham, 37 (16.3%) from Kampong Speu, and 10 (4.4%) resided in Phnom Penh. The attack by rabid animals was "provoked" in 41 (18.1%) and unprovoked in 186 (81.9%) cases. In the 186 unprovoked attacks, the animals appeared healthy in one and ill in 185 (99.5%) cases, which led the patient to bring the head of the attacking dog to the National Reference Center for Rabies at IPC.

Table 1 Vaccine and rabies immunoglobulin (RIG) use data from the Rabies Prevention Center, Institut Pasteur du Cambodge (rpc@ipc), and other identified sites of rabies postexposure prophylaxis (PEP)

	PEP referrals in 2012	RIG doses	RIG type
Site 1 (rpc@ipc)	20,610	591	ERIG
Site 2	1	1	HRIG
Site 3	0 (refer to IPC)	0 (refer to IPC)	0 (refer to IPC)
Site 4	647	5	Unknown
Site 5	Unknown	27	HRIG
Site 6	800	17	ERIG
Other	Unknown Estimated ~10,000, pre- and/or postexposure prophylaxis	Unknown Estimated ~100	HRIG

ERIG = equine rabies immunoglobulin; HRIG = human rabies immunoglobulin; IPC = Institut Pasteur du Cambodge.

All 227 documented exposures from confirmed rabid animals were bites. Of these, 143 (63.0%) were Grade II (no bleeding) and 84 (37.0%) were deep bites (Grade III according to WHO classification). The number of bites was one in 2 cases (0.9%), two in 196 (86.3%), three in 19 (8.4%), four in 8 (3.5%), and more than five in 2 cases (0.8%).

Of the 227 attacks by a confirmed rabid animal documented at rpc@ipc, 92 (40.5%) were located on the foot, 31 (13.7%) on the hand, and 29 (12.8%) were located on the legs. Of these 227 persons, 224 (98.7%) received immunoglobulin. The three persons who did not receive immunoglobulin had received one, three, and five vaccine sessions, respectively. One was a 30-year-old female (Grade II bite to the foot), the second a 22-year-old male (Grade III to the leg), and the third was a 9-year-old female living in Phnom Penh (Grade III to the foot). They were all seen within 24 hours of the exposure and discontinued PEP for personal reasons.

Since 2011, all persons exposed to a confirmed rabid dog were systematically followed up by telephone after 6 months. There were no reported fatalities after PEP in 2012, with or without immunoglobulin. The two adult patients exposed to rabies who received no ERIG were alive and well at their 6-month follow-up. The third patient, a child, could not be followed up, which is frequent in the highly mobile Phnom Penh residents.

Rabies PEP at Other Centers in Cambodia

Outside of rpc@ipc, an estimated total of 53,400 vaccine doses (0.5 mL) and 200 immunoglobulin doses were used in Cambodia in 2012. The internet survey identified five centers other than rpc@ipc, which may be able to provide rabies PEP in Cambodia: one governmental

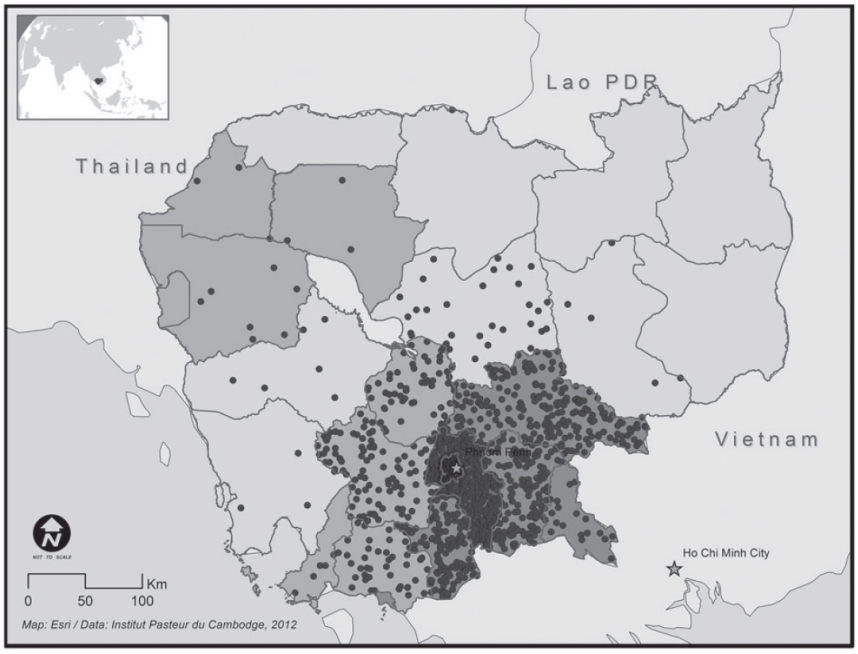

Figure 1 Map of Cambodia showing the number of patients referred or self-referred for rabies postexposure prophylaxis by province of residence, Rabies Prevention Center, Institut Pasteur du Cambodge (rpc@ipc), 2012.

Table 2 Estimated wholesale costs (in US$) associated with vaccine and rabies immunoglobulin (RIG) for rabies postexposure prophylaxis (PEP), Cambodia, 2012

	Vaccine wholesale costs			RIG wholesale costs		Total wholesale cost for recommended Grade III PEP[9]	
	Low vaccine cost range	Average vaccine cost	High vaccine cost range	Low cost ERIG	High cost ERIG	Minimum cost	Maximum cost
Wholesale price per 1 mL dose	US$6.40	US$7.87	US$8.90	US$20.00	US$35.00	—	—
Wholesale price per two 0.1 mL	US$2.56	US$3.15	US$3.56	US$20.00	US$35.00	—	—
Full course of four IM doses	US$25.60	US$31.48	US$35.60	US$20.00	US$35.00	US$45.60	US$70.60
Full course of four double ID doses*	US$10.24	US$12.59	US$14.49	US$20.00	US$35.00	US$30.24	US$49.49

ERIG = equine rabies immunoglobulin; IM = intramuscular; ID = intradermal.
*Modified Thai Red Cross regimen of two 0.1 mL ID doses per session, for a total of four sessions.[9]

institution in Phnom Penh, two Embassy clinics in Phnom Penh, one renowned private health care center in Phnom Penh, and one reference hospital in Siem Reap (Table 1). All five offered rabies vaccine: in 2012, the two largest centers had provided PEP to approximately 800 and 647 cases, respectively; a third center had managed no cases and a fourth had managed one PEP case, while the last was unaware as postexposure patient files were mixed in with pre-exposure files. Three of the centers offered RIG, one of these intermittently, based on availability, but at no cost. Two used human RIG (HRIG), one used ERIG, and one was unaware. The large center with 800 PEP had administered 17 doses of RIG in 2012 while the center with mixed files had administered 27 doses. There is no information in centers outside rpc@ipc on systematic follow-up at 6 months.

Cost of Vaccines and Immunoglobulin

The average wholesale price in 2012 for one dose of the seven available vaccines licensed in Cambodia was US$7.90 (median US$8.10, range US$6.40–US$8.90). The wholesale price for one dose of ERIG ranged between US$20.00 and US$35.00 (Table 2).

Discussion

Rabies vaccine and immunoglobulin are less used in Cambodia and are accessible at IPC and to some extent at some international clinics or reference hospitals. All except one center are located in Phnom Penh, the capital of Cambodia. Rabies vaccine alone may be accessible in some private clinics elsewhere in Cambodia, but usually at a high cost.

At least more than 9% of the patients referred to rpc@ipc with Grade III injuries also received ERIG (40 IU/kg body weight[13,14]) in 2012. This percentage remains low in view of WHO recommendations,[9] although it is higher than that in many developing country settings.[15,16] At IPC, RIG is usually administered because of confirmed laboratory diagnosis of rabies in a biting dog's head or because of a severe bite to the face and/or hand, especially in children. When documented in other centers, this percentage of RIG use varied between 2 and 77%.

In injuries other than bites, patients' wounds and/or immediately surrounding tissue are routinely infiltrated with anesthetics in the emergency room,[17] with steroids in dermatology,[18] or with tetanus immunoglobulin. In many training sessions throughout Cambodia during the past years, one of the coauthors (LS) was told repeatedly by clinicians being initiated to PEP use that they found great technical similarities with performing local anesthesia and that they were very ready to use RIG to infiltrate wounds. What then are the obstacles to RIG use in Cambodia?

According to the clinicians, one obstacle is the inability to calculate the dose according to body weight. Improvements in the prescription leaflet could help solve this issue.

A second obstacle is the perception that ERIG often causes severe adverse reactions (SAR), despite the fact that 4,608 doses of ERIG were used from 2000 to 2012, inclusive, at rpc@ipc with no reported adverse reactions and that ERIG-SAR are considered rare by WHO experts[19]; in fact, so rare that WHO no longer recommends a skin test prior to administering ERIG.[9]

The third, main obstacle to RIG use in Cambodia seems to be its high cost, which is to be added to the cost of vaccination. Expense has also been identified as a key obstacle in other countries.[20–23] The wholesale cost is the cost at which health care teams purchase the vaccine, therefore not including added costs for transport, storage, infrastructure, personnel, and injection materials. This wholesale cost for a full course of rabies vaccination alone was estimated at between US$10 and US$36 in 2012, depending on whether ID or IM protocols are used. ERIG is in limited supply and available at a wholesale cost of between US$20 and US$35 per dose. A Cambodian farmer's monthly salary is between US$60 and US$80.

The first consequence of these costs—to which the cost of transportation must be added—is that even the ERIG used at rpc@ipc remains too expensive for most Cambodians, especially those residing in rural areas where most of the potentially infective dog bites occur.[24] The vaccine and immunoglobulin are therefore heavily subsidized at rpc@ipc and in some cases offered free of charge. A second consequence of these high costs is that centers that do not have a high enough demand for rabies PEP are hesitant to store expensive vaccines and RIG, which may expire before use, and defer the use of intradermal injection of 0.1 mL doses from 0.5 mL or 1 mL vials, letting the remnant go to waste. A third consequence of this high cost and low access to RIG is that travelers cannot be sure of finding local access to timely and adequate PEP after a dog bite.[25–28] Travelers to high-endemic areas such as Cambodia are therefore strongly encouraged to undergo pre-exposure vaccination or seek expert advice, as per recommendations from France and other countries.[9,29–32] Should they be victims of a dog bite, they would not need to interrupt their travel as they would only require a booster shot of vaccine within a few days, but there would be no need for RIG, which in practice is accessible at a reasonable cost only in Phnom Penh. Additionally, some of the rabies vaccines used in unregulated sites in Southeast Asia may be counterfeit.[33]

Most dogs in Cambodia are owned. The patients referred to rpc@ipc after a dog bite in 2012 declared knowing the owner of the biting dog in 19,705 (98%) cases. None of the animals were vaccinated against rabies (rpc@ipc data, Institut Pasteur du Cambodge). If it is to meet its ASEAN commitment to eliminate rabies by 2020,[4–7] Cambodia must solicit international stakeholders and funders to provide or subsidize canine and human vaccines, at least initially. Laws must be passed to enforce vaccination of dogs. State-subsidized, pre-positioned stocks of human vaccine and immunoglobulin in bite management centers would extend the rabies prevention centers network. Support from Institut Pasteur du Cambodge for staff training, cold chain, and quality control would contribute to create well-positioned satellite centers in the provinces, thereby increasing geographic and financial accessibility to timely and effective PEP and reducing the risk of human rabies deaths in Cambodia.

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Declaration of Interests

The authors state that they have no conflicts of interest to declare.

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Part 3: Improving geographical access to timely and adequate rabies PEP in Cambodia

An evidence-based epidemiological strategy for optimal positioning of peripheral rabies prevention centers in Cambodia

7. Rabies Postexposure Prophylaxis (PEP) Noncompletion after Dog Bites: Estimating the Unseen to Meet the Needs of the Underserved

7.1 Introduction

We analyzed the characteristics of patients documented in the Institut Pasteur du Cambodge (IPC) database who did not complete their postexposure prophylaxis (PEP) regimen of their own accord to estimate the number of persons who would most benefit from a rabies prevention center being positioned near their district of residence.

7.2 Method

The successive steps of the method are detailed in Table 5.

We extracted the characteristics of patients who referred to IPC for PEP during the years 2009-2013. Postexposure protocols considered complete were based on WHO recommendations and the regimen developed by the Thai Red Cross (TRC). We used protocol noncompletion after a bite by a potentially rabid dog as a proxy for difficulties or impossibility in accessing timely and adequate PEP, after excluding patients who terminated PEP early due to recommendations (patient previously vaccinated, biting dog survival, biting dog that had tested rabies-negative…). The association of various factors with the risk of noncompletion was studied through univariate and multivariate logistic regression models. In addition, a boosted regression tree model was used to allow non-linear associations for characteristics of the distance between district of residence and IPC. Whatever the model used (logistic regression or boosted regression tree), quality of the prediction of the probability of noncompletion was assessed through the area under the receiver operating curve associated to this prediction. Districts contributing ≤ 5 patients to the database were excluded, as the number of noncompleters was anticipated to be ≤ 1.

Table 5: Summarized analytical strategy to identify rabies PEP geographical coldspots

Step/Objective	Method
1. Constituting the dataset	• Extraction of the 2009-2013 dataset from the Institut Pasteur du Cambodge (IPC) PEP patient database; • Exclusion of patients who terminated PEP early as per medical team recommendations (pre-exposure vaccination, dogs tested negative or which survived…);
2. Complementing the dataset re. difficulties to reach IPC rabies clinic	• 159 district health centers were contacted throughout the country to document: – Distance from Phnom-Penh: Euclidean distance, distance by road, in travel time, travel costs (return trip); – Months corresponding to annual flooding and rice harvests
3. PEP noncompletion and non-geographic factors	• Identification of patients who did not complete the full recommended PEP ("PEP noncompleters") • Univariate then multivariate logistic regression analysis to identify all non-geographic factors associated with PEP noncompletion (<5 sessions to June 2012; <4 sessions after that). • Computation of adjusted noncompletion odds ratio for each variable.
4. PEP noncompletion and district of residence	• Multivariate logistic model exploring the association between district of residence and PEP noncompletion, after adjustment for the non-geographic factors identified in step 3; • Computation for each district of an adjusted noncompletion odds ratio.
5. Expected noncompleter caseload in each district	• Using each district's noncompletion odds ratio (step 4) to approximate relative risk[105,106]; • Computation of the attributable risk percent[107] of noncompletion. • Multiplication of that attributable risk by the estimated number of persons bitten by dogs in each district. • Provides the absolute number of persons who do not complete PEP protocols in each district which would theoretically be avoided by positioning a rabies clinic in that district. $RI_{district\ (i)} = ARP_{ex} * N_{bitten} = ((RR - 1) / RR) * (incid_{bites} * pop_{district})$ *Where ARP_{ex} = attributable risk percent exposed; N_{bitten} = number of dog bite victims expected annually in the district; RR = relative risk; $incid_{bites}$ = annual incidence of dog bites extrapolated to* district [67]*; $pop_{district}$ = 2008 population of the district* [108]*.*
6. Documenting a distance threshold associated with noncompletion	• Boosted regression tree model to explore the association between characteristics of travel from the district center to Phnom Penh (identified in Step 2) and PEP noncompletion after adjustment for non-geographical risk factors associated with noncompletion (Step 3); • Leading to the estimation of a travel threshold beyond which PEP noncompletion increases significantly.
7. Identifying underserved areas	• Mapping of the rabies index for each district in each province, while attributing values 0 : – To Phnom-Penh (where IPC is located); – To six distant and sparsely populated provinces contributing ≤ 5 patients to the study; – To all the districts located within the distance identified as a threshold for increased PEP noncompletion (step 6); • Analysis using the Polygon Continuity method[109] and the Getis and Ord Gi* statistic[110] to identify clusters of districts characterized by a high Rabies Index (high noncompletion clusters).

We computed the attributable risk percent using the relative risk[107] - approximated by the odds ratio of noncompletion as the probability was low (<10%)[105,106] - associated with each district, as produced by the model. This attributable risk percent was then multiplied by the population of each district and the incidence of dog bites[67] was computed to measure population impact and was named the rabies index (RI). This is the absolute number of noncompleted PEP regimens which would theoretically be avoided by positioning a rabies clinic in that district. The RI for each district were then mapped and the Polygon Continuity conceptualization method[109] followed by the computation of the Getis and Ord Gi* statistic[110] was used to identify not only districts with a high noncompletion burden but clustering of high-RI districts, near which positioning a peripheral rabies center would likely have most impact.

7.3 Results

During the years 2009 to 2013, inclusive, the 100,660 patients referred to IPC after exposure to a potentially rabid dog were male in 52% of cases with a mean age of 21 (SD 18.7 years) and a median age of 13 (IQR 6-32). Patients resided in 18 of Cambodia's 24 provinces.

The PEP regimen was considered non-completed by patients in 7,814 (7.8%) of cases. The risk of noncompletion was higher during rice harvest and increased during the study. It was higher for years after 2010, when the delay before referral was > 2 days, when the patient was an adult aged 15 to 49, when the dog had been put down or lost to follow-up, or finally when the prescribed regimen was a full PEP protocol (4 or 5 sessions, depending on the year studied). The risk was lower when patients were females or when the biting dog appeared sick or was confirmed rabid. Some districts were associated to a particularly high percentage of non-completers, such as the distant and sparsely-populated province of Preah Vihear, and conversely to lower noncompletion in the districts of Kandal province, neighboring Phnom Penh. Among various models evaluated, that based on distance by road in kilometers had the highest area under the receiver-operating curve (0.883). This enabled us to identify a threshold associated with a marked increase in noncompletion, for a distance by road of approximately 150 km. After calculating the rabies index for each district based on statistically significant odds ratios obtained earlier, mapping of district clusters identified two significant populated areas which would benefit from peripheral rabies clinics: the cities of Battambang, in the western part of the country, near Thailand; and Prey Veng on the Eastern border with Vietnam.

7.4 Discussion and conclusion

Overall, vaccine PEP noncompletion in this very large data set was approximately 8% and increased very significantly during the rice harvest, during which Cambodians must make a cruel choice between preserving themselves or their loved ones against the risk of rabies and their family's source of revenue for the coming year. Some districts are in themselves associated with a very high risk of noncompletion, with a very marked distance threshold of 150 km by road from the rabies clinic at IPC, Phnom Penh.

To obtain a more precise estimate of the effectiveness of positioning two peripheral rabies prevention clinics in Cambodia, we therefore used not a measure of risk but rather a measure of impact by extrapolating this risk of noncompletion to all populations, including those which had not referred to our center.

Finally, the final results of our study is a map of district clusters in which the theoretical number of people who would complete their vaccine PEP would be the highest if a rabies clinic was positioned there. The population of the largest cluster situated to the east of Battambang will have to travel

approximately 60 km instead of 180 km to Phnom Penh, and that of the cluster located near the 150-km threshold will travel approximately 120 km instead of 150 km. This is also true of patients from prey Veng who will travel 110 km to Kampong Cham instead of 150 km to Phnom Penh. The provinces of Battambang and its four neighboring provinces had in 2008 a total population of 2.04 M, 15.2% of the then 13.4 M population in Cambodia[108].

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7.5 Article 2

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Practice of Epidemiology

Rabies Postexposure Prophylaxis Noncompletion After Dog Bites: Estimating the Unseen to Meet the Needs of the Underserved

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Postexposure prophylaxis (PEP) prevents human rabies and is accessible in Cambodia principally in Phnom Penh, the capital. Timely, affordable access to PEP is a challenge for the mainly rural population. We aimed to identify districts independently associated with PEP noncompletion to position frontline vaccination centers. We analyzed the 2009–2013 database at the Rabies Prevention Center at the Institut Pasteur du Cambodge, Phnom Penh. Logistic regressions identified nongeographic determinants of PEP noncompletion as well as the districts that were independently associated with noncompletion after adjustment for these determinants. The influence of distance by road was estimated using a boosted regression-trees model. We computed a population attributable fraction (rabies index (RI)) for each district and developed a map of this RI distribution. A cartographic analysis based on the statistic developed by Getis and Ord identified clusters of high-RI districts. Factors independently associated with noncompletion were patients' district of residence, male sex, age 15–49 years, initial visit during rice harvest, the dog's status (culled or disappeared), and a prescribed PEP protocol requiring more than 3 PEP sessions (4 or 5). Four clusters of high-RI districts were identified using this analytical strategy, which is applicable to many vaccination or other health services. Positioning frontline PEP centers in these districts could significantly widen access to timely and adequate PEP.

access; dogs; epidemiology; medically underserved area; observance; postexposure prophylaxis; rabies; vaccine

Abbreviations: IPC, Institut Pasteur du Cambodge; PEP, postexposure prophylaxis; RI, rabies index; RPC, Rabies Prevention Center; WHO, World Health Organization.

Human rabies after an animal bite is prevented by immediate wound cleansing and antisepsis followed by timely and adequate postexposure prophylaxis (PEP) (1). Pioneered by Louis Pasteur in 1885 (2), PEP entails the intramuscular or intradermal administration of rabies vaccine, with or without rabies immunoglobulin (1). Although rabies PEP is available in Cambodia, most Cambodians face daunting obstacles to the access of timely, adequate, and affordable PEP in the rural setting (3). A model of rabies deaths in Cambodia has been used to estimate a total of 810 human rabies deaths in 2007 (95% confidence interval: 394, 1,607), for an estimated incidence of 5.8/100,000 (95% confidence interval: 2.8, 11.5), the highest published worldwide (4). As efforts are renewed to control dog-mediated rabies (5), we sought to identify factors associated with PEP noncompletion in data collected by the Rabies Prevention Center (RPC) at the Institut Pasteur du Cambodge (IPC), in Phnom Penh, to optimally position frontline centers and improve geographical access to PEP for underserved Cambodians.

METHODS

Since 1998, the RPC at IPC staff have prospectively entered data on patients and dogs for every initial and follow-up patient visit using a standardized questionnaire (>21,000 referrals each year). Some patients bring the biting animal's severed head to IPC for testing at the National Reference Center for Rabies (Virology Unit, IPC). Brain samples are screened by direct fluorescent test using a fluorescent lyophilized, absorbed

antirabies nucleocapsid conjugate (Bio-Rad, Marnes-la-Coquette, France) (6). We extracted an anonymized version of the RPC at IPC database. Factors studied included sex, age, province/district of residence, and the date of referral. Exposure characteristics included time elapsed before referral, type of exposure, anatomical site of main wound, and World Health Organization (WHO) bite category (1). Further characteristics included whether the attack was provoked; the animal's behavior, status at patient referral, and ownership; and laboratory results if the animal's head was brought to IPC for testing. The RPC at IPC staff administers WHO-recommended PEP protocols (1). Prior to June 6, 2012, the recommended PEP was based on a Thai Red Cross intradermal protocol of 5 sessions (2 intradermal doses at days 0, 3, 7, and 1 intradermal dose at days 28 and 90) if the animal died, was put down, was lost, or tested rabies-positive. After June 6, 2012, the full protocol was shortened to 4 sessions of 2 intradermal vaccine doses (1). The protocol was considered nonobserved/noncompleted if it was terminated by the patient before the fifth ("full PEP" before June 6, 2012) or the fourth session (beginning June 6, 2012), unless: 1) the animal was alive at day 10 (stop after the third session); 2) the patient had been previously immunized as per vaccination records (stop after the second, booster session); or 3) the dog's head tested negative (discontinue after the first session).

We interviewed the head of each of Cambodia's 159 district health centers by telephone. We documented the distance by road (kilometers), travel time (minutes), and estimated cost (in riels; US $1 = 4,000 riels) from the district center to Phnom Penh, as well as the months of flooding and rice harvest. Stung Treng, Mondulkiri, Ratanakiri, Otdar Meanchey, Pailin, and Kep provinces were excluded because their districts contributed less than 5 patients, a number too low to be further analyzed.

Determinants were described and compared between rabies PEP completers and noncompleters (Wilcoxon test for quantitative variables, χ^2 for percentages, and χ^2 for trend for years). Patients' ages were categorized into 3 classes (in years: <15, 15–49, ≥50) and date of referral into a binary variable (during/after the time at which 75% of districts have harvested rice paddy fields). Variables explored for association with noncompletion were year and time of year of referral, time elapsed between bite and patient referral, sociodemographic variables (age, sex), characteristics of the exposure (type, severity, and anatomical site of the exposure), characteristics of the dog (behavior, ownership, and rabies confirmation status), and characteristics of PEP sessions. Variables associated with noncompletion in univariate analysis with a significance level of $P < 0.2$ were all included in a multivariate logistic regression model. Factors independently associated with a P value of <0.05 were retained in the model through a backward selection, using Wald's test. A second logistic regression model was developed in the same way, this time to quantify the association of district of residence with PEP noncompletion, adjusted for all nongeographical factors independently associated with noncompletion in the previous multivariate model. The model's discriminating power was evaluated by computing the area under the receiver operating characteristic curve. The relationship between the distance to the PEP center (measured as Euclidian distance, distance by road, travel time, or travel cost to Doun Penh district, where IPC is located) and the probability of 4-session ("full") PEP noncompletion was suspected to be nonlinear. Boosted regression-tree models fitting complex nonlinear relationships (7) were therefore used to assess independently the role of each distance measurement variable adjusted for nongeographical variables (7). The model with the highest area under the receiver operating curve (using distance by road) was retained and provided a full-PEP noncompletion cutoff for distance to the RPC at IPC.

The risk of noncompletion in a district was quantified by computing an attributable risk percent among those exposed (8). This percentage estimates the proportion of people who are referred but do not complete PEP after a bite by a potentially rabid dog. It is computed using a relative risk (8). The models used, however, compute an odds ratio of noncompletion. The odds ratio was deemed to approximate the relative risk well because the probability of noncompletion was low (<10%) (9, 10). The ensuing "rabies index" (RI) is an absolute number, computed by multiplying the attributable risk percent among the exposed by the estimated number of dog bite victims, itself a product of the population of the district (11) and the annual incidence of dog bites documented by another prospective IPC study (12). The RI is therefore a measure of impact that reflects the theoretical PEP-noncompleter caseload in a given district that is underserved by the current centralized RPC, and who in theory would better access and complete timely and adequate PEP if the regimen were made available in or close to that district:

$$\begin{aligned} \mathrm{RI}_{district\,(i)} &= ARP_{ex} \times N_{bitten} \\ &= ((RR-1)/RR) \times \ (incid_{bites} \times pop_{district}), \end{aligned}$$

where ARP_{ex} is the attributable risk percent among the exposed, N_{bitten} is the number of dog bite victims expected annually in the district, RR is relative risk, $incid_{bites}$ is the annual incidence of dog bites extrapolated to district (12), and $pop_{district}$ is the 2008 population of the district (11).

Finally, we mapped the RI for all districts in Cambodia. The RI value of 0 was attributed to Phnom Penh, districts located within the noncompletion distance cutoff, and districts not included in the analysis due to low representation in the database. We modeled the spatial relationship between districts using the polygon-contiguity conceptualization method (13). A cartographic "hot spot" analysis of polygons representing districts was conducted based on the Getis-Ord Gi* statistic to assess spatial clustering of the RI indicator (14). Clusters of high-RI districts surrounded on all sides by other high-RI districts were considered significant hot spots when their Gi* P value was <0.05 (z score > 1.96).

During the period covered, RPC at IPC data was entered using EpiInfo (US Centers for Disease Control and Prevention, Atlanta, Georgia). Statistical results were obtained using Stata, version 13 (StataCorp LP, College Station, Texas), and R, version 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria). Spatial data and statistics were mapped using ArcMap, version 10.0 (ESRI, Redlands, California).

This study received approval from the Cambodian National Ethics Committee for Human Research.

RESULTS

The number of patients documented in the 2009–2013 RPC at IPC database and the data flow are shown in Figure 1.

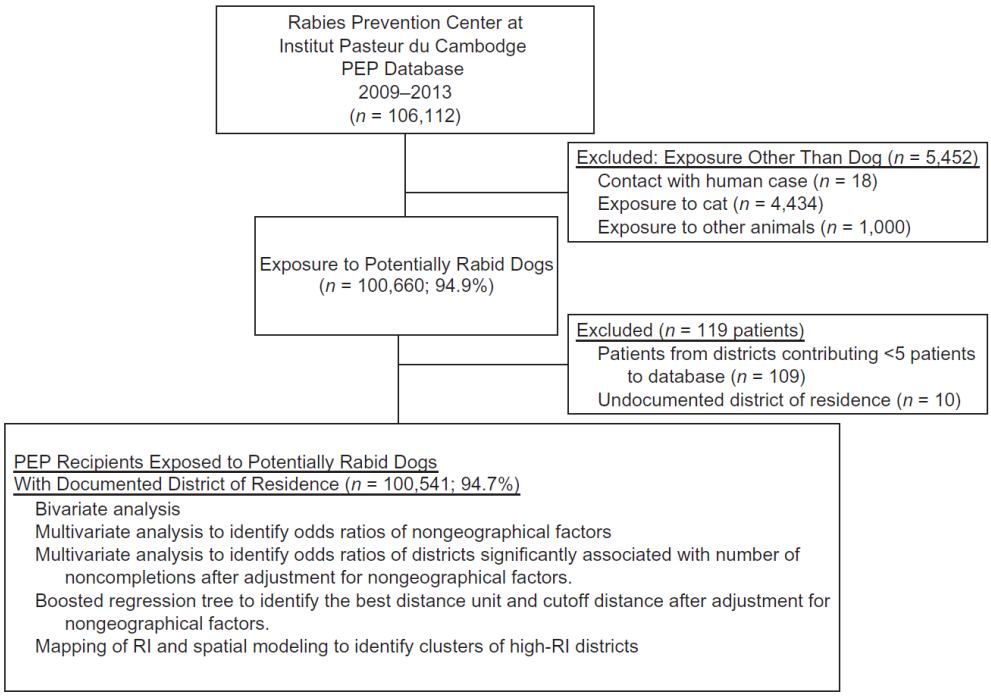

Figure 1. Study data flow and analyses on patients referred or self-referred for postexposure prophylaxis (PEP) after an exposure to a potentially rabid dog, Rabies Prevention Center at the Institut Pasteur du Cambodge, Phnom Penh, Cambodia, 2009–2013. RI: rabies index.

The descriptive study analyzed a total of 100,660 patients exposed to potentially rabid dogs who were referred to IPC for PEP after a WHO bite category II or III exposure (Table 1). Patients were male in 52.4% of cases, and their mean age was 21.2 (standard deviation, 18.7) years (median, 13; interquartile range, 6–32). They resided among 18 of Cambodia's then 24 provinces. Overall, 92,104 (91.5%) patients resided in 5 provinces located in or around the capital, Phnom Penh, which itself contributed 44,573 (42.3%) patients. The mean delay between exposure and first injection was 1.64 (standard deviation, 2.01) days, with a median of 1 day (interquartile range, 1–2). The exposures, among which 74,590 (74.0%) were WHO category II and 26,070 (26.0%) were WHO category III exposures, all required PEP. The dog's owner was identified in 99,648 (99.0%) cases, and the dog appeared ill in 2,163 (2.1%) cases. Exposures occurred to lower extremities in 65,323 (64.9%) cases and to the head and neck in 6,178 (6.1%) cases. During the study period, 1,864 biting dog's heads were brought to the center and analyzed at IPC's virology unit, confirming rabies in 1,098 (58.9%) dogs. Prescribed PEP was considered noncompleted in 7,814 (7.8%) of all cases, including 7,618 (7.6%) bites (Table 1).

The year (χ^2 for trend $P < 0.001$) was significantly associated to PEP noncompletion, as was the number of prescribed PEP sessions ($P < 0.001$) (Table 1); 1,030 (25.0%) of intended 4-session PEP and 4,744 (58.4%) of intended five-session regimens (the RPC reference protocol to June 2012) were not completed. Compared to completers, PEP noncompleters tended to present later after a bite and to be more frequent among male patients. There was no statistically significant difference between PEP completers and noncompleters in terms of WHO bite category. Among PEP noncompleters, the dog's owner was less often identified and the dog had more often died, been put down, or been lost to follow-up. Finally, the proportion of patients who brought a head for testing was higher among PEP noncompleters.

PEP noncompletion was significantly higher during rice harvest, rising to 8.7% in November–April compared with 7.1% the rest of the year ($P < 0.001$). The rainy season (including floods) was not associated with noncompletion. Noncompleters traveled significantly greater distances, had longer transportation times, and had higher transportation costs than PEP completers (Table 1).

Factors independently associated with PEP noncompletion were year, time elapsed before PEP, age 15–49 years, referral during the rice harvest, the fact that the dog had been put down or lost to follow-up, and a prescribed regimen requiring more than 3 sessions (4 or 5 sessions) (Table 2). Factors independently associated with PEP completion were being of female sex, the biting dog appearing sick, and laboratory-confirmed rabies in the biting dog's head.

The district of residence was significantly associated with PEP completion after adjustment for the nongeographic characteristics mentioned above. Among Cambodia's 132 districts, 24 (18.2%) were associated with a significant odds ratio of

Table 1. Characteristics of Patients Referred or Self-Referred for Postexposure Prophylaxis After Exposure to a Potentially Rabid Dog, Rabies Prevention Center at Institut Pasteur du Cambodge, Phnom Penh, Cambodia, 2009–2013

Characteristic	Total (n = 100,660)		Noncompleted Protocols (n = 7,814)			Completed Protocols (n = 92,846)			P Value
	No.	Median (IQR)	No.	%	Median (IQR)	No.	%	Median (IQR)	
Patient Characteristics									
Year									<0.001[a]
2009	21,068		1,461	6.9		19,607	93.1		
2010	21,064		1,473	7.0		19,591	93.0		
2011	19,340		1,351	7.0		17,989	93.0		
2012	19,491		1,653	8.5		17,838	91.5		
2013	19,697		1,876	9.5		17,821	90.5		
Time elapsed, days									<0.001[a,b]
Median (IQR)		1.0 (1.0–2.0)			1.0 (1.0–3.0)			1.0 (1.0–2.0)	
≤1 day	62,591		3,985	6.3		58,606	93.6		
>1 day and ≤2 days	17,779		1,508	8.5		16,271	91.5		
>2 days	20,290		2,321	11.4		17,969	88.6		
Age, years									<0.001[b,c]
Median (IQR)		13.0 (6.0–32.0)			15.0 (7.0–33.0)			13.0 (6.0–32.0)	
Missing	22		2	9.1		20	90.9		
<15	52,175		3,823	7.3		48,352	92.7		
15–49	37,155		3,096	8.3		34,059	91.7		
≥50	11,308		893	7.9		10,415	92.1		
Sex									<0.001[c]
Male	52,761		4,265	8.1		48,496	91.9		
Female	47,899		3,549	7.4		44,350	92.6		
Type of exposure									<0.001[c]
Scratch or lick on nonintact skin	210		196	93.3		14	6.7		
Bite	100,450		7,618	7.6		92,832	92.4		
WHO bite category									0.108[c]
I	0		NA			NA			
II	74,590		5,850	7.8		68,740	92.2		
III	26,070		1,964	7.5		24,106	92.5		
Anatomical site of main exposures									
Head and neck	6,178		513	8.3		5,665	91.7		0.101[c]
Torso	8,928		623	7.0		8,305	93.0		0.004[c]
Upper limbs	21,185		2,024	9.6		19,161	90.4		<0.001[c]
Lower limbs	65,323		4,757	7.3		60,566	92.7		<0.001[c]
Genitals	247		14	5.7		233	94.3		0.218[c]
Type of attack									<0.001[c]
Provoked	29,656		2,111	7.1		27,545	92.9		
Unprovoked	71,004		5,703	8.0		65,301	92.0		
Biting dog's behavior									<0.001[c]
Sick	2,163		674	31.6		1,489	68.8		
Healthy	98,497		7,140	7.2		91,357	92.8		
Ownership									<0.001[c]
Owned dog	99,648		7,476	7.5		92,172	92.5		
No identified owner	1,012		338	33.4		674	66.6		

Table continues

Table 1. Continued

Characteristic	Total (n = 100,660)		Noncompleted Protocols (n = 7,814)			Completed Protocols (n = 92,846)			*P* Value
	No.	Median (IQR)	No.	%	Median (IQR)	No.	%	Median (IQR)	
Dog's status									<0.001[c]
Put down	10,666		4,559	42.7		6,107	57.3		
Died	812		313	38.5		499	61.4		
Lost	1,623		877	54.0		746	46.0		
Alive	87,558		2,064	2.4		85,494	97.6		
Missing	1		1	0.0		0	0.0		
Number of recommended PEP sessions									<0.001[a]
Missing	9		7	77.8		2	22.2		
1	765		0	0.0		765	100.0		
2 (boosters)	1,277		22	1.7		1,255	98.3		
3	86,368		2,016	2.3		84,352	97.7		
4	4,121		1,030	25.0		3,091	75.0		
5	8,120		4,744	58.4		3,376	41.6		
Result of rabies testing									<0.001[c]
Negative	765		0	0.0		765	100.0		
Positive	1,098		248	22.6		850	77.4		
Not tested/ inconclusive	98,797		7,566	7.7		91,231	92.3		
District Characteristics									
Rice harvest season									<0.001[c]
No	57,036		4,040	7.1		52,996	92.2		
Yes	43,624		3,774	8.6		39,850	91.3		
District distance to Doun Penh District, Phnom Penh									
Missing	137		16	0.2		121	0.1		
Euclidian distance, km		19.0 (5.6–48.0)			36.0 (14.4–64.4)			16.0 (5.6–46.1)	<0.001[b]
Road distance, km[d]		30.0 (8.0–60.0)			57.0 (22–95)			25.0 (8–60)	<0.001[b]
Travel time, minutes[d]		60.0 (30.0–90.0)			60.0 (40.0–120.0)			60.0 (30.0–90.0)	<0.001[b]
Travel costs, riels[d]		10,000 (7,000–15,000)			10,000 (7,000–15,0000)			10,000 (8,000–20,000)	<0.001[b]

Abbreviations: IQR, interquartile range; PEP, postexposure prophylaxis.
[a] χ^2 for trend.
[b] Wilcoxon test to compare medians.
[c] χ^2 test.
[d] For 100,523 (99.9%) patients.

noncompletion, the highest being Tbaeng Meanchey district, Preah Vihear province (Table 3). Districts with statistically significant odds ratios for PEP noncompletion were generally the most distant from IPC and had lower population densities. The area under the receiver operating curve analysis showed that the multivariate model had very good discrimination (88.0%).

Distance by road, travel time, and travel costs were highly correlated. The model found to have the highest area under the receiver operating curve was based on distance by road in kilometers; this variable was thus maintained in the boosted regression-trees model. The representation of noncompletion dependence with distance by road after adjustment for nongeographic factors confirmed nonlinearity and estimated a cutoff of approximately 150 km, beyond which the noncompletion percentage increased significantly for intention-to-treat by full 4- or 5-session protocols (Web Figure 1, available at https://academic.oup.com/aje).

Web Figure 2 presents a map of districts by RI as well as borders for clusters of underserved, high-RI districts (Gi* z scores > 1.96). This cluster analysis identified 2 large clusters of underserved districts: At least 1 frontline rabies prevention center can thus be optimally positioned in Bat

Table 2. Odds Ratios for Nongeographical Factors Associated With Rabies Postexposure Prophylaxis Noncompletion Following Logistic Regression (Excluding District of Residence), Rabies Prevention Center at Institut Pasteur du Cambodge, Phnom Penh, Cambodia, 2009–2013

Variable	Unadjusted			Adjusted		
	OR	95% CI	*P* Value[a]	OR	95% CI	*P* Value[a]
Year			<0.001			<0.001
2009	1.0	Referent		1.0	Referent	
2010	1.0	0.93, 1.1		1.1	1.0, 1.3	
2011	1.0	0.9, 1.1		1.1	1.0, 1.2	
2012	1.2	1.2, 1.3		4.4	3.9, 5.0	
2013	1.4	1.3, 1.5		10.0	8.9, 11.3	
Time elapsed, days			<0.001			<0.001
≤1	1.0	Referent		1.0	Referent	
>1 and ≤2	1.4	1.3, 1.4		1.1	1.0, 1.2	
>2	1.9	1.8, 2.0		1.1	1.1, 1.2	
Age, years			<0.001			<0.001
<15	1.0	Referent		1.0	Referent	
15–49	1.1	1.1, 1.2		1.2	1.1, 1.3	
≥50	1.1	1.0, 1.2		1.1	1.0, 1.2	
Sex			<0.001			0.006
Male	1.0	Referent		1.0	Referent	
Female	0.9	0.9, 0.9		0.9	0.9, 1.0	
Rice harvest season			<0.001			<0.001
No	1.0	Referent		1.0	Referent	
Yes	1.2	1.2, 1.3		1.2	1.2, 1.3	
Type of exposure						
Bite	0.006	0.003, 0.01	<0.001	0.02	0.01, 0.05	<0.001
Scratch/lick on nonintact skin	96.6	63.7, 146.6	<0.001	1.13	0.20, 6.53	0.888
WHO bite category			0.10			0.290
II	1.0	Referent		1.0	Referent	
III	1.0	0.9, 1.0		0.96	0.89, 1.03	
Anatomical site of exposure[b]						
Head and neck	1.1	1.0, 1.2	0.11	0.87	0.67, 1.12	0.282
Torso	0.9	0.8, 1.0	0.004	0.90	0.78, 1.04	0.156
Upper limb	1.3	1.3, 1.4	<0.001	0.99	0.91, 1.05	0.570
Lower limb	0.8	0.8, 0.9	<0.001	0.95	0.87, 1.04	0.297
Genitals	0.7	0.4, 1.2	0.211	NA[c]	NA[c]	
Type of attack			<0.001			0.047
Unprovoked	1.0	Referent		1.0	Referent	
Provoked	0.9	0.8, 0.9		0.9	0.9, 1.0	
Biting dog behavior			<0.001			<0.001
Healthy	1.0	Referent		1.0	Referent	
Sick	5.8	5.3, 6.4		0.49	0.4, 0.6	
Ownership			<0.001			0.093
Owned dog	1.0	Referent		1.0	Referent	
No identified owner	6.2	5.4, 7.0		0.86	0.72, 1.02	

Table continues

Table 2. Continued

Variable	Unadjusted			Adjusted		
	OR	95% CI	*P* Value[a]	OR	95% CI	*P* Value[a]
Dog's status			<0.001			<0.001
Put down	30.7	29.0, 32.6		1.7	1.4, 2.1	
Died spontaneously	26.4	22.8, 30.6		1.3	1.0, 1.7	
Lost	44.7	40.2, 49.6		2.3	1.9, 2.9	
Alive	1.0	Referent		1.0	Referent	
Rabies virological result in dog head[d]			<0.001			<0.001
Positive	3.5	3.04, 4.05		0.47	0.38, 0.59	
Not tested/inconclusive	1.0	Referent		1.0	Referent	
No. of visits needed for PEP completion			<0.001			<0.001
2	0.7	0.5, 1.1		0.4	0.3, 0.7	
3	1.0	Referent		1.0	Referent	
4	13.9	12.8, 15.1		4.1	3.4, 5.041	
5	58.8	55.3, 62.6		99.5	79.9, 123.9	

Abbreviations: CI, confidence interval; NA, not applicable; OR, odd ratio; PEP, postexposure prophylaxis.
[a] Likelihood ratio test.
[b] Because patients were often injured at several anatomical sites, the reference category for each wounded anatomical site is the absence of a wound at that anatomical site.
[c] Not included after unadjusted analysis because $P > 0.2$.
[d] Heads tested negative and 1-visit recommendations were removed from the model due to the absence of noncompletion in these subjects.

Table 3. Odds Ratios for Postexposure Prophylaxis Noncompletion According to District of Residence, Before and After Adjustment for Nongeographical Factors, Rabies Prevention Center at Institut Pasteur du Cambodge, Phnom Penh, Cambodia, 2009–2013

Province	No. of Patients in Database for Province	District[a]	No. of Patients in Database for District	Unadjusted			Adjusted		
				OR	95% CI	*P* Value[b]	OR	95% CI	*P* Value
Rabies Prevention Center, Phnom Penh	38,875	Doun Penh	3,695	1.0	Referent		1.0	Referent	
Kandal	22,562	Khsach Kandal	4,346	2.89	1.06, 3.73	<0.001	1.36	1.06, 1.73	0.014
Kampong Cham	11,134	Memot	55	4.8	3.5, 6.8	0.1	0.30	0.09, 0.98	0.04
		Srey Santhor	171	3.43	2.60, 4.54	<0.001	0.68	0.48, 0.97	0.03
Takeo	5,982	Kaoh Andet	12	1.22	0.29, 5.08	0.78	0.14	0.03, 0.75	0.02
Prey Veng	5,791	Me Sang	516	6.02	4.51, 8.02	<0.001	1.76	1.22, 2.53	0.002
		Kampong Trabaek	355	5.37	3.85, 7.51	<0.001	1.61	1.05, 2.46	0.03
Kampong Speu	4,110	Thpong	284	8.34	6.00, 11.58	<0.001	1.80	1.13, 2.854	0.01
Kampong Chhnang	2,358	Boribo	89	2.66	1.83, 3.86	<0.001	0.60	0.37, 0.95	0.03
Svay Rieng	851	Kampong Rou	92	4.62	2.51, 8.53	<0.01	2.44	1.12, 5.33	0.02
Battambang	285	Moung Reussei	42	6.61	3.00, 14.57	<0.01	4.30	1.39, 13.25	0.01
		Thmor Kol	18	8.03	2.61, 24.73	<0.01	5.26	1.38, 20.01	0.01
Pursat	247	Sampov Meas	56	6.87	3.47, 13.59	<0.01	3.80	1.45, 9.97	0.01
Banteay Meanchey	114	O Chrov	30	18.78	8.83, 39.71	<0.01	6.71	2.34, 19.22	<0.01
Preah Vihear	36	Tbaeng Meanchey	17	8.64	2.78, 26.88	<0.01	9.30	2.24, 38.55	0.002

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Districts not appearing in this table are those associated with an OR found not significantly different from 1 after adjustment.
[b] Wald test.

Dambang (Battambang) and Prey Veng provinces to serve these districts as a public health priority, while other districts closer to Phnom Penh can benefit from information before frontline centers can be opened secondarily (Web Figure 2).

DISCUSSION

Canine-mediated rabies is almost 100% fatal, but timely and adequate PEP is almost 100% effective. Long-term rabies control and elimination can only be obtained by immunizing dogs (15). There is, however, a lag time between the initiation of dog vaccination programs and consequent reduction in human rabies deaths (16, 17). During that time, lives can be spared by timely, adequate, and affordable PEP—a highly effective intervention, especially in rabies-endemic settings (18, 19). This is a considerable challenge, especially in the rural endemic areas of developing countries where most rabies cases occur. In 2008, 80.5% of the Cambodian population (67% of whom lived in 10 southeastern provinces) was rural (11).

Few studies have been carried out on the determinants of PEP noncompletion (20). Our global percentage of noncompleters (7.8%) is lower than that reported at Queen Saovabha Memorial Institute in Bangkok, Thailand (22.6%), but remains high in Cambodia where canine rabies is widespread and highly enzootic (20). In several IPC studies, rural Cambodians were keenly aware of the transmission mode and lethality of rabies (21). The rabies burden in dogs was reduced in Thailand, perhaps reducing perceived risk and completion (22). Last, rabies PEP is subsidized at IPC but not cost-free. This may encourage patients to return and complete a PEP protocol for which they have paid up front in full.

Our study enabled us to better identify factors independently associated with rabies PEP noncompletion in Cambodia: Male patients aged 15–49 years and those exposed to a dog put down or lost to follow-up were more likely to discontinue the PEP regimen, likely because they resided in distant rural villages. Noncompletion was especially frequent during rice harvest, when rural Cambodians face the stark choice of ensuring livelihoods for the year or spending precious money and time to return to Phnom Penh and continue PEP. Such assessments may need to be repeated regularly, as shown by the significant associations with year studied and changes in epidemiology, road network, and road use. Some districts close to Phnom Penh were also independently associated with noncompletion but not because of distance: These areas will likely benefit from reinforced messages rather than from positioning another center nearby.

Beyond a certain distance, however, information alone will not reduce noncompletion. Making PEP accessible throughout Thailand greatly reduced reported human rabies cases (17, 23). Our study identified districts where rabies PEP outposts can be positioned with the greatest benefit for Cambodians: Distance was identified as an independent noncompletion factor for full PEP protocols, with a cutoff estimated at 150 km by road. Furthermore, the number of patients residing in various Cambodian districts expected to discontinue protocols early (assuming a generalizable incidence of bites and applying it to population figures) gave an indication of underserved areas.

Allocating scarce resources to a large number of people at medium risk of a disease that is not transmissible from person to person is more effective than allocating them to a small number of people at high risk (24). The choice was made to position rabies prevention centers in areas with the highest anticipated impact. To do so, we computed a composite index, postulating that: 1) the odds ratio for noncompletion was a good approximation of the relative risk, which is considered to be the case when the event is infrequent (<10%) (9, 10); 2) the number of expected PEP regimens was a product of the population size and the incidence of dog bites, the latter having been extrapolated from an earlier study to all Cambodian districts; and 3) the 2008 population census data were a good approximation of the population and its distribution across districts for the study period. Our original strategy can be used as a complement to seroepidemiologic studies and registries to identify underserved areas and quantify needs (25, 26).

Our study may have biases and limitations. One, extrapolating a single district's dog-bite incidence rate to all districts in Cambodia may misestimate the true rate. This, however, is the best available estimate, and 80% of the Cambodian population lives in similar settings. Two, roads progressively improve in Cambodia: The difficulty in reaching IPC may change over time. Conversely, road traffic, travel times, and prices continue to increase. We accounted for this by examining only data from recent years. Three, our models did not adjust for patients' socioeconomic status, which is not routinely collected at IPC. Distance, rural living, and being affected by the rice harvest, however, are good proxies for low socioeconomic status, which does not differ greatly across populations in rural Cambodia (in 2008, the third-quartile monthly disposable income per capita in rural Cambodian households was around US $40.00 (27)). Four, the database cannot differentiate between patients bitten by "observable" dogs at baseline that were alive after 10 days (no need to return for a fourth session) and patients bitten by dogs that died during the first week and thus who should have returned for a fourth session. Among the 87,558 patients bitten by an observable dog at baseline, 2,064 (2.4%) were noncompleters. In the absence of veterinary quarantine services for dogs, this shortcoming cannot be addressed. Five, errors may occur during data entry in a busy rabies prevention center. This may have occurred, but random entry errors are unlikely to have affected results due to the large number of patients included in this analysis. Systematic errors that may have occurred due to variables being documented by the same team throughout the study period are unlikely to be linked to whether PEP was completed by the patient. Finally, our strategy is based on examining noncompletion in people who were referred to the RPC, but it cannot account for people who were not, especially from western Cambodia. Although this is unavoidable, our strategy covers most Cambodian districts (home to 95.4% of the Cambodian population, including Phnom Penh) and compensates by mapping districts' expected number of PEP noncompleters.

As in the seminal work by Wald on damaged yet surviving planes (28), attempting to measure what we do not see rather than what we see is challenging. Aside from a very recent study (29), previously published attempts to map underserved areas or populations have been overwhelmingly based on estimates of distance and travel times (30–32). Using a real-world database to map underserved district clusters based on the Getis-Ord Gi* statistic (14) enabled us to identify neighboring areas with a high RI, thereby reflecting a more reliable estimate

of the high number of anticipated noncompleters and improving likely cost-effectiveness. This innovative strategy can be applied to any public health measure, including screening or care services, as long as: 1) the risk of disease is known or can be extrapolated throughout the country; 2) the risk is below 10%; 3) district populations are reasonably well documented; and 4) the patient databases analyzed are near-exhaustive for most of the territory being examined. It can also be added to the toolkit for rabies elimination as WHO leads renewed efforts to eliminate dog-mediated, human rabies deaths (5, 33).

Cambodian authorities have committed to eliminating canine rabies by 2030 (34). This ambitious task will take time, during which improving access to timely, affordable, and adequate PEP would spare human lives in Cambodia. IPC will assist in optimally positioning frontline sites to improve geographical access to PEP. Access can also be improved by reducing the duration and doses of PEP protocols to keep costs down. As a research agency of the Cambodian Ministry of Health, IPC is working to attain this objective at no additional risk to patients.

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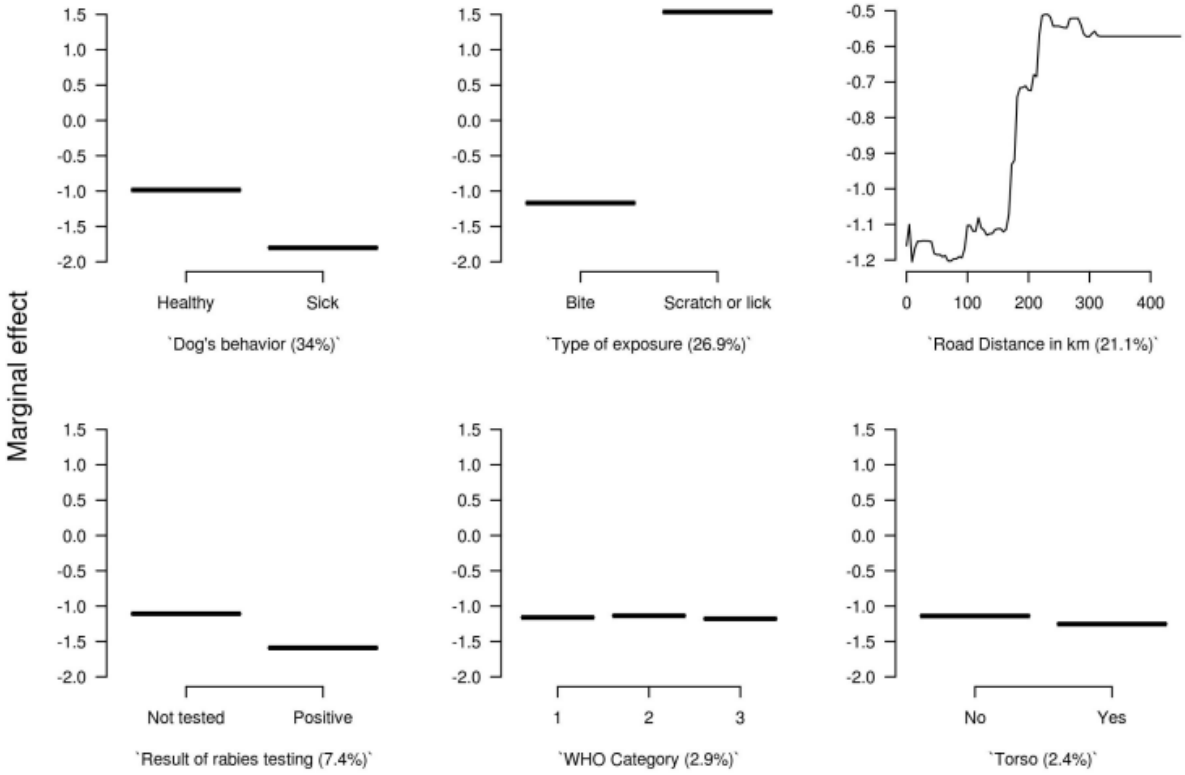
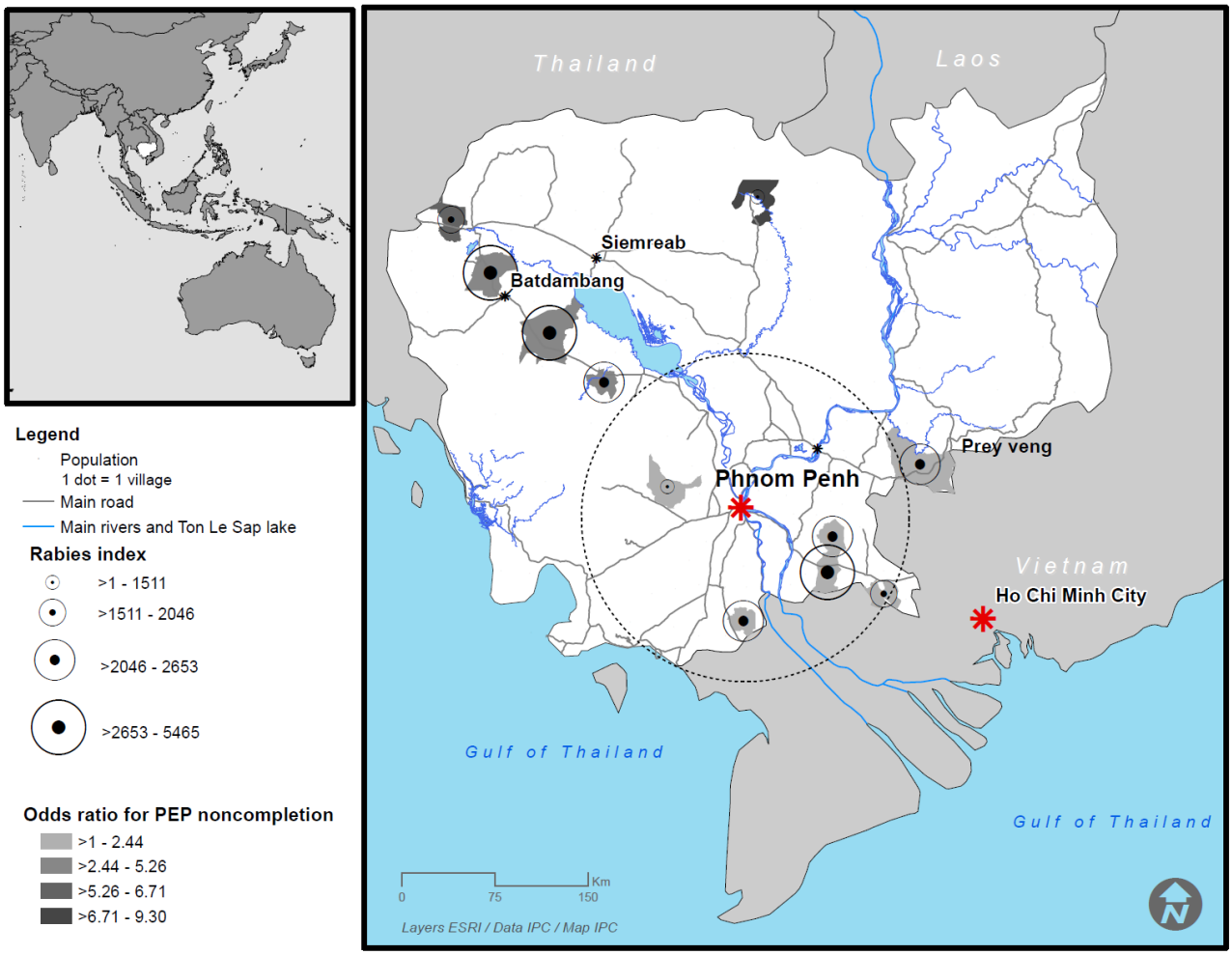
Figure 3: Boosted regression tree and partial dependences graphs showing influence >1% in probabilities after adjustment for other factors and 150-km distance from Phnom-Penh's Doun Penh District, the distance threshold for noncompletion of intended four-session post-exposure prophylaxis protocols, Rabies Prevention Center at Institut Pasteur du Cambodge (rpc@ipc) database 2009-2013. Phnom Penh. Cambodia.



Parameters used were as follows: a tree complexity (number of nodes) of 3, an initial number of trees set at 50, a learning rate of 0.001 and a bag fraction of 0.5. The y variable (outcome) was PEP noncompletion and the explanatory variables (x) were non-geographical factors which presented a p-value <0.2 in the initial univariate analysis mentioned above.

Figure 4: Clusters of underserved districts identified by a high burden of PEP noncompleters (Rabies index) and 150-km distance from Phnom-Penh's Doun Penh District, Rabies Prevention Center at Institut Pasteur du Cambodge, 2009-2013.



Part 4: Improving financial access to timely and adequate rabies PEP in Cambodia

A clinical and epidemiological observation of an "in natura" dataset among PEP noncompleters

8. Clinical Outcome after Complete *vs.* Abridged Intradermal Post-Exposure Prophylaxis (PEP) Following Exposure to Rabies-Confirmed or Rabies-Suspected Dogs, Cambodia, 2003-2014: Intradermal Rabies PEP can be Abridged

8.1 Introduction

Rabies post exposure prophylaxis (PEP) is currently considered to be accessible to less than 5% of persons bitten by a potentially rabid dog[71]. Aside from bite victims' lack of knowledge of post-bite management, the obstacle is first and foremost due to difficulties in accessing PEP, which in Cambodia is available only in the capital for most bite victims[71]. The issue of barriers in geographical access has been addressed in our first article[111]. There remains, however, the major difficulty of financial access in a country where the monthly income *per capita* is around US$40. Abridging PEP to a duration of one week instead of one month could have a major impact on rural populations' access to timely and adequate PEP in Cambodia. We therefore sought to document the clinical effectiveness of PEP in patients who terminated it early (three sessions during the first week) compared to patients who received a full course of PEP (four sessions over a one- month period or more) after referring to the rabies prevention clinic at Institut Pasteur du Cambodge (IPC) between 2003 and 2014, inclusive, after a bite by a potentially rabid dog.

8.2 Method

The successive steps of the method are detailed in Table 6.

Data for 2003-2014, inclusive, pertaining to sociodemographic characteristics, bite lesions and dog status and direct fluorescence antibody rabies testing (DFAT) results[59] were extracted from the IPC patient database which prospectively documents PEP referrals since 1998.

Table 6: Summarized analytical strategy to investigate the association between abridged (3-sessions) post-exposure prophylaxis (PEP) and probable rabies deaths

Step/Objective	Method
1. Constituting the initial IPC dataset	• Extraction of the 2003-2014 dataset from the Institut Pasteur du Cambodge (IPC) PEP patient database; • Exclusion of: – Patients who were already vaccinated against rabies; – Patients bitten by dogs tested negative or untested dogs which survived (no rabies exposure).
2. Documenting patient outcome ≥6 months after PEP	• Callback using the mobile number in the database. • If failure, contact of district health center then village chief then patients (in Khmer) to: – Confirm patient identity; – Obtain informed consent; – Document clinical outcome. • If the patient was unidentified by the Village Chief: considered lost to follow-up.
3. Identification of rabies deaths	• If the patient was identified but had died. • Verbal autopsy by semi-structured interview of next-of-kin by MD in Khmer using a standardized form; • Determination of most likely cause of death (rabies or other cause) by external rabies expert committee, blinded to our assessment.
Performance of PEP, overall and by PEP sessions category	
4. Rabies deaths: overall description and per number of PEP sessions received	• Overall percentage of rabies deaths among persons bitten by rabies-confirmed and by confirmed and/or sick-looking dogs with exact binomial confidence intervals. • Number of rabies deaths, stratified by: – PEP sessions; – RIG received; – Biting dog status.
5. Testing the association between the number of PEP sessions received and rabies death	• Estimating the probability of an early death belonging to the 3-sessons group by: – The observed proportion of patients who received 3 sessions in the dataset, excluding early deaths; – Multivariate logistic regression to assess the association between the number of sessions received and all characteristics documented in the initial dataset to compute probability using coefficients. • Computation of unilateral Fisher test with midpoint p value for each hypothesis of early deaths allocation; • Weighted unilateral Fisher test with midpoint p value, taking into account the uncertainty regarding allocation to the number of PEP sessions category of patients who died of rabies between sessions 3 and 4 (early deaths).
All subsequent work considers the most likely early death allocation hypothesis	
6. Determination of the characteristics of the association between rabies deaths and number of PEP sessions	• Computation of the unconditional odds ratio estimated through maximum likelihood and of its 95%CI obtained by inversion of the initial Fisher test. • Estimation of power of unilateral Fisher test using a theoretical odds ratio close to that estimated.
7. Association of other factors than number of PEP sessions received with rabies death	• Univariate and multivariate logistic regression models of rabies deaths according to characteristics of patients in the initial dataset to estimate the association between PEP completion (3 *vs.* 4+ sessions) and rabies death after adjustment for all other factors.

All patients bitten confirmed rabid dogs or untested but sick-looking dogs were included in the study, except previously vaccinated patients, those bitten by dogs tested negative or which survived (therefore non-rabid). All patients were contacted by telephone after at least six months, first using the telephone number documented in the database. As Cambodians often change mobile phone SIM cards and cannot

be reached by mail, the health center of each patient's district of residence were contacted to obtain the telephone number of the patient's village chief. Calling the village chief enabled the team to verify whether someone of the patient's name resided in the village and if so obtain that person's telephone number or arrange a telephone interview. During that subsequent interview in Khmer, we verified the patient's identity, obtained informed consent to participate, and documented clinical outcome. Patients who could not be identified by calling their number directly or through the village chief were considered lost to follow-up. We excluded Phnom Penh residents from the dataset as initial efforts showed that these were urban residents were extremely mobile and therefore extremely difficult to trace back.

In case the patient had died, a verbal autopsy was conducted in Khmer by telephone with next-of-kin by one rabies-experienced Medical Doctor through a semi-structured interview using a standardized questionnaire. All verbal autopsy reports where reviewed by a group of outside experts blinded to our own conclusions to attribute probable cause of death to rabies or to a cause other than rabies.

The overall percentage of rabies deaths despite PEP among persons bitten by rabies-confirmed and by confirmed or untested sick-looking dogs was computed, with exact binomial confidence intervals. Analysis first bore on all patients to estimate the percentage of death from rabies, stratified by number of PEP sessions, RIG received and the dog status. For each patient who died from rabies after the third session but before the fourth session (early deaths") we evaluated the probability that the patient would have completed PEP, using: 1/ the proportion of PEP terminated after 3 sessions only observed in the database (after excluding patients who died early of rabies) and 2/ the probability for each early death of belonging to the 3-sessions category, as estimated through multivariate logistic regression of belonging to either sessions group (3 *vs.* 4+) as a function of all patient characteristics documented in the dataset.

The unilateral Fisher mid-p value testing the association between PEP noncompletion and rabies death was performed for each possible series of allocations (for instance 0, 1, 2, … , all early deaths allocated to the 3-sessions group, the remaining cases being allocated to the 4+ sessions group). The overall, weighted Fisher unilateral mi-p value was then obtained by multiplying the unilateral Fisher mid-p value of each allocation hypothesis by the probability of occurrence of that hypothesis, as described above.

Subsequent analyses were restricted to the most likely early deaths allocation hypothesis. The unconditional odds ratio was estimated through maximization of the likelihood and its mid-point-adjusted 95%CI was obtained by inverting the test[112]. Mid-point-adjusted power was estimated *post-hoc*, using an odds ratio with a theoretical value close to that estimated. A logistic regression model then explored the association between various factors and rabies deaths, and another association between receiving 3 or 4+ sessions PEP and the occurrence of a rabies death. A statistical significance threshold of 5.0% was used for all tests.

8.3 Results

In all, 3,838 patients received Vero cell-based vaccine PEP between 2003 and 2014 after a bite by a confirmed rabid or an untested but sick-looking dog. After excluding 520 Phnom Penh residents and 513 patients lost to follow-up, the final data set included 1,739 patients bitten by confirmed rabid dogs and 1,066 patients bitten by an untested but sick-looking dog, for a total of 2,805 followed-up patients. Our study identified 24 deaths from non-rabies causes (road accidents, drowning, old age, chest pain, ascites, etc.) including 2 who died before 6 months' follow-up, as well as three probable rabies deaths. The percentage of rabies deaths after a bite by a rabid dog despite PEP is therefore 3/1,739 (1.7 per 1000). It is 3/2,805 (one per 1,000) despite PEP after a bite by a confirmed rabid or untested but sick-looking dog ("any dog").

Two rabies deaths out of three were early deaths. The midpoint p value of the weighted unilateral Fisher test was 0.0959. Taking into account the individual probabilities derived from the logistic model for the two early deaths to be allocated to the 3-sessions group, the overall weighed Fisher p value was 0.0961.

In univariate analysis, Category III bites and principal bites to the head/neck were associated with a higher risk of rabies. All three deaths all suffered Category III bites. Consequently, no multivariate logistic model of rabies death could be derived (no convergence), making adjustment on independent characteristics impossible.

The unadjusted odds ratio of the association between receiving three sessions only and dying of rabies was 6.30 but the confidence interval included 1. It was lower (OR=4.44) and similarly non-significant in patients bitten by untested but sick-looking dogs. The power of the unilateral Fisher exact test, however, was estimated at 49% in the dataset of patients exposed to confirmed rabid dogs.

8.4 Discussion and conclusion

In summary, 99.83% of patients bitten by confirmed rabid dogs did not die of rabies with a follow-up of at least six months after PEP.

Our study concluded that there was no element in favor of a difference in percentage of rabies deaths amongst those who received three PEP sessions only compared to those who received four sessions or more. Statistical power, at 49%, requires careful follow-up of patients with suspect rabies exposure when implementing this IPC regimen.

The article was submitted in August 2018 to *Vaccine* and has been accepted with revisions.

8.5 Article 3 (revised draft after reply to reviewers)

Intradermal Rabies Post-exposure Prophylaxis Can Be Abridged with No Measurable Impact on Clinical Outcome in Cambodia, 2003-2014

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Declaration of Interest

Dr. Philippe Buchy is a former Head of Virology at Institut Pasteur du Cambodge and is currently an employee of GSK Vaccines

Abstract

Rabies causes 60,000 deaths worldwide annually. Rabies post-exposure prophylaxis is highly effective but often geographically and financially beyond reach in endemic developing countries. We conducted a retrospective study on clinical outcome at ≥6 months in 3,318 Cambodians who received intradermal Vero cell vaccine post-exposure prophylaxis after a bite by a rabid or sick-looking but untested dog in 2003-2014. An external expert panel examined verbal autopsy reports to identify rabies deaths. 1,739 (93.65%) persons bitten by rabid- and 1,066 (72.96%) bitten by sick-looking but untested dogs were traced and 513 were lost to follow-up. Among the former, 1,591 (91.49%) and 129 (7.42%) patients referred for 4+ and 3 post-exposure prophylaxis sessions, respectively. Three persons died of probable rabies so that the overall percentage of survival was 99.83% (95% exact confidence interval: 99.49 – 99.96%) in post-exposure prophylaxis recipients bitten by confirmed rabid dogs. No significant difference was found in survival among patients who received 3 *vs.* 4+ sessions (with or without rabies immunoglobin). The power of the study, however, was limited. The current four sessions / one month intradermal regimen can be reduced to a three sessions / one week at no detectable added risk to patients, with the limitation of study power at 49%. A clinical follow-up system should be adopted by rabies prevention centers, especially to monitor implementation of an abridged course. The Institut Pasteur in Cambodia regimen will improve vaccine equity by treating 33% more patients with available doses, reduce direct cost of vaccination, transportation and other indirect costs to vaccinees.

Funding: Institut Pasteur;

Key words: Rabies; Post-exposure prophylaxis; Survival; Intradermal; Abridged regimens, Vaccine

Introduction

Rabies is an infectious meningoencephalitis syndrome caused by rabies virus (RABV), the prototypical virus of the *Rhabdoviridae* family, *Lyssavirus* genus. Human rabies cases worldwide overwhelmingly follow a bite by a rabid dog (1). After a bite, RABV transmission depends on various factors including bite severity and anatomical site and is inconstant. The outcome of clinical human rabies is almost always fatal, with only few known exceptions (2,3). Rabies causes an estimated 60,000 cases worldwide every year and is emerging in previously unaffected areas of the World (1,4–6). Rabies is almost 100% preventable by timely and adequate post-exposure prophylaxis (PEP).

Cambodia is a Southeast Asian country with a population of approximately 15 million, 75% of which reside in rural areas (7,8). Dogs in Cambodia are overwhelmingly owner dogs. The incidence of human rabies in Cambodia, however, is among the highest worldwide due to a very high dog-to-human ratio and dog bite incidence as well as difficulties to access PEP (9–11).

The current dose-sparing, highly effective Thai Red Cross (TRC) PEP protocol entails four sessions of intradermal (ID) injection of two 0.1 mL vaccine doses over one month (12). The cost of the protocol generates important issues, limiting access to PEP in the rural areas of developing countries where populations are most at risk of rabies (1,11,13). An intradermal abridged protocol of three sessions over one week would reduce vaccine use per patient by 25%, allowing the treatment of 33% more patients with the same quantity of vaccine. It would improve equity and coverage, and reduce direct and indirect (travel…) costs to patients (14).

The objective of this RESIST (Rabies Elimination Support through Integrative Science and salvage Therapy) study was to document the clinical effectiveness of an intradermal abridged PEP protocol, comparing outcome in people who discontinued PEP after only three sessions *vs.* after the recommended four or more (4+) sessions of two 0.1 mL ID injections using Vero cell-based vaccine after a bite by confirmed rabid or by sick-looking but untested dogs, whether the latter were put down or escaped (henceforth referred to as "sick-looking or untested dogs").

Methods

Rabies center database

Clinical and epidemiological characteristics of patients referred each year for PEP at Institut Pasteur du Cambodge (IPC) are prospectively entered into a database since 1998. The same, trained team of clinicians and nurses systematically document patients. This team enters patients' self-declared village and district of residence, sociodemographic characteristics, dog and bite characteristics – including exposure category (12) - into an electronic form using EpiData (EpiData Association, Odense, Denmark) also used to monitor PEP sessions. An extraction of the database documents 203,519 Cambodian residents referred or self-referred to IPC after a dog bite over a period of 12 years, from January 1st, 2003 to December 31st, 2014, inclusive. Patients who were identified as previously vaccinated against rabies in IPC vaccination records or through the initial PEP interview were excluded from the analysis.

Virological testing at IPC

Heads of biting animals brought by about 2% of bite victims are also tested at IPC. After craniotomy and brain extraction, tissue samples are taken from Ammon's horn and the medulla oblongata (15). As per WHO/OIE recommendations, samples undergo a direct fluorescent antibody test (DFAT) using an adsorbed, lyophilized anti-rabies nucleocapsid conjugate (#357-2112, Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer's instructions (15). Results are usually available on the day of the initial referral.

Rabies PEP at IPC

Since 2003, all rabies PEP for WHO Category II / III exposures (12) at IPC use Vero cell vaccine (Verorab®, Sanofi, France) administered to each deltoid intradermally (ID) using 25-gauge needles. In 2012, the full protocol changed from five to four sessions (Days 0, 3, 7 and 28) of two 0.1 mL ID doses as per WHO 2010 recommendations (12). Due to chronic and severe shortages, available equine rabies immunoglobulin (ERIG) is administered in priority in case of positive DFAT results in the biting animal, sick-looking dogs or bites to the face, even in Category II exposures (11).

Callback at IPC

All previously unvaccinated patients bitten by rabid or sick but untested, non-surviving dogs who received ID Vero-cell based vaccine at IPC (with or without ERIG and whether or not they had returned for all prescribed PEP sessions of their own accord) between 2003 and 2014, inclusive, were eligible for the study. These were systematically traced back at least six months after initiation of PEP. Phnom Penh residents were excluded as

initial attempts failed to identify any of these highly mobile persons. Since Cambodians cannot be reached by mail, the IPC team contacted the head of the health center nearest to the patient's village by phone to obtain the mobile phone number of the village chief. The latter was contacted to verify whether he knew of village resident of that name. If so, a telephone meeting was arranged with the person or kin, during which consent to participate was obtained (in Khmer). Patient identity and bite characteristics were verified without prompting. Outcome (patient dead, alive or lost to follow-up) was systematically entered in the database.Verbal autopsy

If the patient was identified and traced back but had died, a verbal autopsy was conducted in Khmer by telephone with next of kin by an IPC doctor experienced in rabies using a standardized semi-structured interview form. All deaths were reviewed by an external panel of three rabies experts from India and Thailand on April 28th, 2017 to determine if they could be attributed to probable rabies as per established case definitions (16). These experts were blind to the fatal cases' PEP status and our own conclusion.

Statistical analysis

Patients' baseline characteristics (sociodemographic, clinical and bite) and dog characteristics (including test results in dog heads when available), patients' PEP protocol completion and clinical outcome were assessed. Categorical variables were described as frequencies and percentages and continuous variables as median and interquartile range.

The overall percentage of rabies deaths among persons bitten by a rabies-confirmed dog or by a confirmed or sick-looking dog ("*any dog*") was computed, with exact 95% binomial confidence intervals (CI).

The proportions of rabies deaths by number of sessions were compared using unilateral Fisher's exact test and its mid-point p value to assess clinical inferiority of 3 sessions compared to 4+ sessions (17). Rabies-attributed deaths that occurred before the date of the fourth session – termed "early deaths" – were not allocated *a priori* to a number of sessions and the various allocation hypotheses were explored. Denote N the number of early deaths. We computed the proportion π of patients in the 3-sessions group in our sample, after excluding the early deaths. We then derived, for k=0,…, N, the probability that k early deaths among N would have been allocated to the three-sessions PEP group if they had not died early. The sum of the Fisher mid-point p value for each value of k (=0,…, N) weighted by their probability provided an overall Fisher p value taking into

account the possible allocations of early deaths. This was done in patients bitten by confirmed rabid dogs and in patients bitten by "*any dog*".

After exclusion of the early deaths, the distribution of each baseline characteristic was compared between the 3 and 4+ sessions groups using Fisher's or Chi-square test for categorical variables and Wilcoxon test for continuous variables. Multiple logistic regression was used to assess the probability for a patient of belonging to the 3-sessions group according to patient's characteristics. The model was then used to compute for each early death the probability to belong to the 3-sessions group and the overall Fisher p value was re-estimated. Subsequent analyses were restricted to the most likely early deaths allocation hypothesis. The unconditional odds ratio was estimated through maximization of the likelihood and its mid-point-adjusted 95%CI was obtained by inverting the test (18). Mid-point-adjusted power was estimated *post-hoc*, using an odds-ratio with a theoretical value close to that estimated.

Fisher's exact test and univariate regression were used to assess the association between each characteristic with rabies among patients bitten by confirmed rabid dogs. A multivariate logistic model was used to identify independent baseline characteristics associated with rabies and to assess the association with 3 *vs.* 4+ sessions, after adjustment for all these independently associated factors. A statistical significance threshold of 5.0% was used for all tests.

The Cambodian National Ethics Committee for Human Research approved the study.

Results

Patients

Between January 1st, 2003 and December 31st, 2014, inclusive, a total of 203,519 patients referred to IPC after a bite by a potentially rabid dog. In total, 3,838 patients had not been previously vaccinated and were bitten by a confirmed rabid or sick-looking but untested dog, excluding surviving dogs (Figure 1). Among these, 520 Phnom Penh residents were excluded from the analysis (Supplementary Table ST1). Among the 3,318 patients living outside Phnom Penh included in the call-back procedure, 513 were lost to follow-up (Supplementary Table ST2): 118/1,857 (6.35%) persons bitten by dogs with confirmed rabies and 395/1,461 (27.04%) bitten by sick-looking but untested dogs, corresponding to a statistically significant difference in loss to follow-up ($p<0.001$).

A total of 2,805 previously unvaccinated patients living outside Phnom Penh who received ID PEP by Vero cell-based vaccine and with at least 6 months' follow-up were therefore included in the study (Table 1): 1,739 (62.00%) were bitten by a dog with confirmed rabies and 1,066 (38.00%) by an untested but sick-looking dog. In all, 2,062 (73.54%) were contacted 12 months or more after PEP (median 31 months; IQR: 31.1-52.7; range 6-151).

Deaths

A total of 27 deaths were documented at least 6 months after PEP by verbal autopsy.

Twenty-four were due to accidents (road accidents, drowning…) or chronic illness (old age, chest pain, ascites, etc.), two occurring in the three months following PEP (one liver cancer and one respiratory failure during pregnancy; both had received RIG and 4+ sessions).

The three remaining deaths were attributed to rabies by the external expert panel (Table 2). Rabies Cases 1 and 2 were bitten on the same day in the same province by different confirmed rabid dogs in different districts. They were referred to IPC on the same day. In total, 144 and 195 other study patients bitten by dogs with confirmed rabies received the vaccine and ERIG lots used in Cases 1-2 and Case 3, respectively, and survived. Case 3's extensive head wound was sutured before referral to IPC for PEP. Cases 1 and 3 died before the planned date of the 4th PEP session (early deaths).

The number of rabies deaths by number of PEP session, with or without RIG and by dog rabid status is detailed in Table 3. In this Cambodian setting, the overall percentage of rabies following ID PEP is therefore 3/1,739 (0.17%; exact 95% CI: 0.03 – 0.50%) after a bite by a dog with confirmed rabies and 3/2,805 (0.10%; exact 95% CI: 0.03 – 0.33%) after a bite by "*any dog*".

Assessing clinical inferiority of 3 sessions compared to 4+ sessions

The probability for patients receiving more than 2 sessions to have referred for 3 sessions only was 127/1,591 (7.39%). The probability that the two early deaths would have both received 3 sessions (Hypothesis 1), that one or the other would have received 4+ sessions (Hypothesis 2) or that both would have received 4+ sessions (Hypothesis 3) were therefore 0.55%, 13.69% and 85.76%, respectively. The sum of the mid-p values for Hypotheses 1-3, each weighed by their likelihood, yielded an overall unilateral Fisher mid-point estimate of 0.0959 (non-significant) when comparing deaths among patients who received 3 or 4+ sessions after being

bitten by a dog with confirmed rabies (Table 4). The overall mid-point p value was 0.1158 (non-significant) for those bitten by "*any dog*" (Supplementary Table ST3).

The distribution of baseline characteristics of patients who received 3 PEP sessions *vs.* those who received 4+ sessions are presented in Table 5 (Table ST4 for patients bitten by "*any dog*"). Based on these data, the individual probabilities derived from the logistic model for the two early deaths to be allocated to the 3-sessions group were 7.23% and 7.34%, respectively, leading to an overall weighed Fisher p value of 0.0961, very close to the initial estimate of 0.0959.

Subsequent analyses consider the most likely hypothesis of the two early deaths occurring in the 4+-sessions group. The unadjusted odds ratio of the association between rabies death and receiving three PEP sessions only was estimated at 6.30 [95% CI: 0.21 - 83.30] for patients bitten by a dog with confirmed rabies and the mid-point-adjusted power was estimated at 49% for a theoretical odds-ratio of 6.50.

Table 6 describes the association between rabies death and baseline characteristics of patients after a bite by a dog with confirmed rabies (Table ST5 for "*any dog*"). In univariate analysis, Category III bites and principal bites to the head/neck were associated with a higher risk of rabies. The odds-ratio for bite category could not be estimated since the three deaths all suffered Category III bites. Consequently, no multivariate logistic model of rabies death could be derived (no convergence), making adjustment on independent characteristics impossible.

Discussion

We describe three probable rabies deaths among 1,739 Cambodians traced at least six months after receiving PEP following a bite by a dog with confirmed rabies or among 2,805 bitten by "*any dog*", with a percentage of PEP recipients alive at 6 months of 99.83% (exact 95% CI: 99.49 – 99.96%) and 99.89% (exact 95% CI: 99.67– 99.97%), respectively. We were unable to demonstrate a statistically significant decrease in survival among patients who received 3 sessions when compared to 4+ sessions (with or without RIG). Adopting a 3-sessions regimen would share rabies vaccine doses more equitably, reducing vaccine use in patients by 25% and treating 33% more patients with the same vaccine quantity. In addition, this would spare patient resources and time spent for travel and loss of daily wages (11,14), as well as reduce patient crowding in high-throughput rabies clinics.

The inoculated RABV and ensuing rabies risk are neutralized by lavage, antisepsis and PEP within hours or days, while RABV remains located at the wound site (16). Protection against rabies after a given bite is linked with short-term neutralization of RABV, not long-term antibody persistence. A three-session / one month pre-exposure intramuscular (IM) vaccination regimen has long been known to be effective (12,19). Three-session ID regimens are included in the 2010 recommendations but these are also pre-exposure and last longer than one week at the time of writing (12). A balance must be carefully struck between the much-desired abridgement of rabies PEP, the number of doses necessary to obtain an early protective immune response and the number of boosters. Patients in this observational study did not undergo serological monitoring due to sheer numbers and lack of funding. However laborious, *in natura* experiments such as our study in a cohort of patients exposed to dogs with confirmed rabies or by sick but untested dogs, stratified by PEP completion and RIG, offer the best real-life data reflecting clinical protection by PEP, especially abridged. A few authors have investigated the effectiveness of one-week PEP regimen with RIG, but only one was conducted in bite victims and none provided outcome data (20–22). Aside from a very limited study documenting five IM PEP non-completers in Puerto Rico (23), our study is the largest to provide data on real-world clinical outcome in persons receiving abridged ID PEP after exposure to confirmed rabid or suspected but untested dogs in a rabies-endemic country. Other strengths of our study are that: data were prospectively collected (except outcome) with low loss to follow-up; an independent expert group attributed the cause of death to rabies; except for two variables ("owner identified" and "ERIG received"), patients who received 3 and 4+ PEP sessions were similar at baseline. Rabies cases die at home in many endemic rural areas but can be confirmed by verbal autopsy when virological diagnosis is lacking (11). The callback system we established to document clinical outcome at six months for this IPC study has become routine. It will be used to guide future improvements in management by detecting protocol failures or deviation or to alert to otherwise ineffective PEP.

Rabies deaths despite PEP are extremely rare (24–26). The probability of death due to rabies that we report among patients with confirmed rabies exposure is one-tenth of that published by Quiambao *et al.* (1.6%) in a smaller real-world cohort of 122 patients despite ID PEP and RIG following a bite by a rabid animal (27). Another study after TRC-ID one-month PEP with RIG found zero deaths among 110 persons bitten by rabies-confirmed dogs (28). In a large study in the Philippines, 3 In 2012, the full protocol changed from five to four

1 (1.68%) of 1,839 rabies cases received PEP, of which eight also received RIG; one died of rabies despite full PEP and RIG (29). Another study conducted in Pakistan documented two deaths among 2,811 intradermal PEP recipients with Category II/III exposure (30). These fatal cases had not completed PEP but the number of sessions completed is not mentioned. Furthermore, the dogs' rabid status in the 2,811 bite victims is undocumented. Such extremely rare rabies deaths are usually attributed to deviations from PEP protocols or direct delivery of RABV into nervous endings (30). Sadly, our study documented three such fatal cases, which must be examined.

The fatal cases in our study suffered several bites to highly innervated areas of the body (head or finger), both factors known to be associated with transmission of rabies despite PEP. Cases 1 and 3 occurred after a short incubation period (19 days in each case) likely related to direct delivery of RABV into nervous endings, despite timely PEP including ERIG. The vaccine and RIG lots used in these patients were found effective by the manufacturer and/or were not associated with death in other confirmed PEP recipients following a bite by a dog with confirmed rabies. The death of Case 3 was highly likely, considering the extensive wounds to the head and unrecommended but necessary suturing. Case 2 is the only one to have died 39 days after ending the protocol after three sessions. Importantly, he had been bitten by a rabid dog in a different district of the same province but was managed for a bite to the head on the same day as Case 1, who also received ERIG and ID vaccine on the same day at IPC. We suspect that these two clustered deaths were therefore most likely due to a clinical management failure such as undetected wounds not being thoroughly infiltrated with ERIG in an extremely busy rabies prevention clinic with a total of 186 rabies PEP administered on that day (31).

Our field study has potential biases and limitations. First, this "natural experiment" may incur information bias by interviewing next-of-kin. The outcome, however, is survival or death and is less prone to bias. Second, verbal autopsies cannot fully replace virological confirmation and may underestimate deaths by misclassifying rabies cases with a paralytic presentation. Verbal autopsy tools, however, are particularly performant in the retrospective documentation of "furious" rabies which account for 80% of cases or more (32,33).

Furthermore, misclassification could have occurred in any PEP session subclass and bias would have been nonsystematic. Third, an additional 129 and 391 Phnom Penh residents bitten by rabid or sick-looking but untested dogs, respectively, were excluded based on early difficulties to trace them back. Unlike other residents, Phnom Penh residents are not tied to land or local industry, are highly mobile with looser social ties,

therefore requiring a great amount of time to be traced, usually unsuccessfully. However, according to the differences existing between Phnom Penh and other residents exposed to rabid dogs (Supplementary Table ST1), the proportion of Category III bites was significantly higher among those residing outside Phnom Penh. Furthermore - as happened for Case 3 - our team would have been informed of suspected rabies deaths, IPC being widely identified as the expert center for rabies in Cambodia. Our strategy to exclude Phnom Penh residents was probably more prone to underestimating the efficacy of PEP in any PEP category, abridged or otherwise. Fourth, follow up was at least six months, potentially missing cases which may have died later. The present study, however, was undertaken between late 2013 and 2016, by which time > 73% of our retrospectively documented patients had over one year's follow-up. Furthermore, rabies incubation is usually shorter than six months (34): In an unrelated 1998-2007 study, the median incubation period in 44 Cambodian human rabies cases – all unvaccinated - was 60 days, with a range of 30 – 100 days (10). In any case, such evaluation bias would have been nonsystematic across PEP session categories. Fifth, loss to follow-up may have omitted patients who died of rabies, underestimating the risk of PEP failure. The loss to follow-up was low - especially among rabies-exposed patients (6.35%) - because our center collects data in a timely fashion, made important efforts to trace back and call patients back after six months starting in 2013 and residents of rural areas often have stable situations. Comparisons between those traced back and those lost to follow-up show that the latter had more frequent risk factors associated with higher rabies transmission risks (Supplementary Table ST2) and longer time elapsed since PEP. Had loss to follow-up led to undetected rabies deaths, these could have occurred in any PEP category, abridged or otherwise. Sixth, the use of ERIG (prioritized in case of confirmed exposure to rabies and/or a bite to the head/neck and/or fingers) may have brought the various vaccine protocols' differences towards the null, overestimating the effectiveness of a 3-sessions regimen. This may be the case, but it would have been unethical to do otherwise. Of note, 53 and 165 patients bitten by confirmed rabid and rabid or untested dogs, respectively, received no ERIG (shortage) and did not develop rabies. Finally, our study suffers from limited statistical power, estimated *post-hoc* at 49%. Ours is the largest series worldwide to our knowledge and denominators are ample but PEP is so effective that deaths were rare, as we were hoping.

Conclusion

Our real-life study could not document a decrease in effectiveness of a three ID sessions / one week PEP regimen of two ID 0.1 mL doses at days 0, 3 and 7 - with or without RIG – compared to the time-proven, highly effective four-session / one-month regimen. As suggested for IM regimens (23), our findings therefore support the abridgment of the TRC to an IPC protocol at no detectable added risk to patients, with the limitation of study power. The World Health Organization endorsed this regimen in its 2018 recommendations as the first one-week and dose-sparing PEP regimen (35). Post-PEP monitoring is continuing at IPC and should be implemented worldwide, especially during the initial phases following the introduction of this regimen. Adopting the IPC regimen will significantly reduce cost for vaccine, repeat transportation and accommodation and other indirect vaccination costs and share vaccine doses more equitably by reducing vaccine use in patients.

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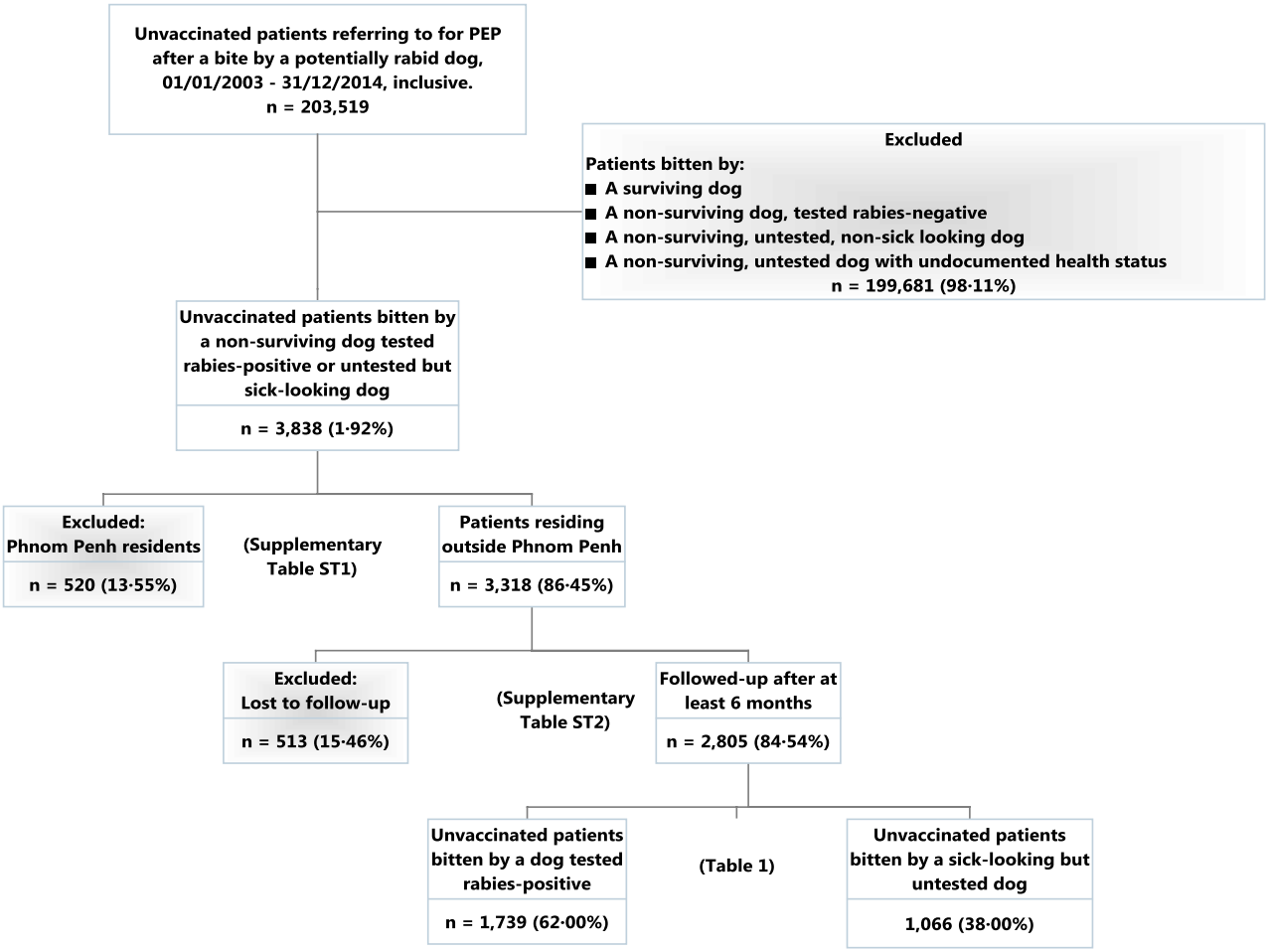
Figure 1: Study data and patient flow, 2003-2014, Institut Pasteur du Cambodge.



Legend: PEP - post-exposure rabies prophylaxis. Vaccination status was documented during the patient interview and by verifying in the IPC database. Dogs' sick appearance was documented based on patients' declaration only and entered into the database.

Table 7: Distribution of selected socio-demographic and baseline clinical characteristics of non-immune patients residing outside Phnom Penh, who received Vero cell-based rabies intradermal post-exposure prophylaxis after a bite by a confirmed rabid or a sick but untested dog (excluding surviving dogs) and not lost to follow-up, 2003-2014, Institut Pasteur du Cambodge.

	Patients bitten by		
	Rabies-confirmed dog (n=1,739)	Sick but untested dog (n=1,066)	« *Any dog* » (n=2,805)
Age (years) – median (IQR)	17 (9-37)	25 (12-45)	20 (10-40)
<15 years old	763 (43.88%)	349 (32.73%)	1,112 (39.64%)
15-65 years old	915 (52.62%)	673 (63.13%)	1,588 (56.61%)
>65 years old	61 (3.51%)	44 (4.13%)	105 (3.74%)
Male	999 (57.45%)	576 (54.03%)	1,575 (56.15%)
Bite category (12)			
Category II	1,218 (70.04%)	870 (81.61%)	2,088 (74.44%)
Category III	521 (29.96%)	196 (18.39%)	717 (25.56%)
Anatomical site of the principal bite*			
Foot/leg	989 (56.90%)	624 (58.54%)	1,613 (57.52%)
Hand	545 (31.36%)	335 (31.43%)	880 (31.38%)
Head / neck	136 (7.83%)	59 (5.53%)	195 (6.95%)
Other bite characteristics			
Number of bite wounds – median (IQR)	2 (2-2)	2 (2-2)	2 (2-2)
One bite wound	129 (7.42%)	121 (11.35%)	250 (8.91%)
Two bite wounds	1,317 (75.73%)	834 (78.24%)	2,151 (76.68%)
Three bite wounds or more	293 (16.85%)	111 (10.41%)	404 (14.40%)
Documented suture◇	14/376 (3.72%)	12/545 (2.20%)	26/921 (2.82%)
Clothes interposed	446 (25.65%)	261 (24.48%)	707 (25.20%)
Documented wound care◇	482/752 (64.10%)	235/652 (36.04%)	717 / 1,404 (51.01%)
Dog status			
Spontaneous bite	1,464 (84.19%)	815 (76.45%)	2,279 (81.25%)
Sick-looking	1,657 (95.28%)	1,065 (99.91%)	2,722 (97.04%)
Identified owner	1,464 (84.19%)	879 (82.46%)	2,343 (83.53%)
N persons bitten – median (IQR)	2 (1-3)	1 (1-2)	1 (1-3)
One person only	848 (48.76%)	621 (58.26%)	1,469 (52.37%)
Two persons	417 (23.98%)	179 (16.79%)	596 (21.25%)
More than two persons	474 (27.25%)	266 (24.95%)	740 (26.38%)
Spontaneous death documented°	128 (7.36%)	115 (10.79%)	243 (8.66%)
Rabies testing	All tested positive	None tested	-
PEP characteristics			
Year of PEP – median (IQR)	2010 (2008-2012)	2013 (2011-2014)	2011 (2008-2013)
Delay before PEP (days) – median (IQR)	1 (0-1)	2 (1-3)	1 (1-2)
Same day (Day 0)	493 (28.35%)	137 (12.85%)	630 (22.46%)
After 1-6 days	1,232 (70.85%)	875 (82.08%)	2,107 (75.12%)
After one week (>Day 6)	14 (0.80%)	54 (5.07%)	68 (2.42%)
ERIG received	1,677 (96.43%)	886 (83.11%)	2,563 (91.37%)
PEP sessions – median (IQR)	5 (4-5)	4 (4-4)	4 (4-5)
1-2 sessions	19 (1.09%)	34 (3.19%)	53 (1.89%)
3 sessions	129 (7.42%)	128 (12.01%)	257 (9.16%)
4 or 5 sessions	1,591 (91.49%)	904 (84.80%)	2,495 (88.95%)
Follow-up			
Delay until callback (months) – median (IQR)	24.25 (6.57-47.33)	30.4 (21.2-46.27)	26.93 (9.7-46.87)
≥ 12 months	1,001 (57.59%)	1,061 (99.53%)	2,062 (73.54%)

* Non-exclusive categories as multiple bites on various anatomical sites are possible; ° The majority of biting dogs were immediately put down, before they could die spontaneously; ◇ These variables were poorly documented. Abbreviations: IQR: interquartile range; PEP: post-exposure prophylaxis; ERIG: Equine rabies immunoglobulin.

Table 2: Details of probable rabies deaths in intradermal post-exposure prophylaxis recipients, 2003-2014, Institut Pasteur du Cambodge

Case	Case 1	Case 2	Case 3
Sex	M	M	M
Age at PEP (years)	37	5	9
Province	Kandal	Kandal	Kandal
Date of bite	17-jul.-2008*	17-jul.-2008*	07-apr-2011
Date of PEP	17-jul.-2008	17-jul.-2008	08-apr-2011
Date death	5-aug-2008	1-sept.-2008	27-apr-2011
Days survived	19	46	19
N sessions completed	3	3	3
ERIG	Yes	Yes	Yes
Dog head	Positive	Positive	Positive
Bite category (12)	Category III	Category III	Category III
Anatomical site	Fingers	Head	Head
Signs	Hypersalivation and contracture	Fever and convulsions	Fever, convulsions, hypersalivation
Expert opinion	Rabies death	Rabies death	Rabies death

* Two patients bitten by two different dogs in two different districts, but first initiated PEP on the same day at Institut Pasteur du Cambodge.

Table 3: Number of patients alive and of probable rabies deaths at least 6 months after a bite by a confirmed rabid or a suspect but untested dog, stratified by number of intradermal post-exposure prophylaxis sessions and equine rabies immunoglobulin received and biting dog status, 2003-2014, Institut Pasteur du Cambodge.

	Confirmed 1 session only		Confirmed 2 sessions only		Confirmed 3 sessions only		Confirmed 4+ sessions only		Received 3 sessions but could be allocated to 3 or 4+ sessions*		Any number of sessions		Total
Probable rabies death	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	
Confirmed rabid dog													
ERIG	5	0	4	0	119	1	1,546	0	0	2	1,674	3	1,677
No ERIG	8	0	2	0	7	0	45	0	0	0	62	0	62
Subtotal	13	0	6	0	126	1	1,591	0	0	2	1,736	3	1,739
Sick but untested dog											0		0
ERIG	10	0	10	0	82	0	784	0	0	0	886	0	886
No ERIG	8	0	6	0	46	0	120	0	0	0	180	0	180
Subtotal	18	0	16	0	128	0	904	0	0	0	1,066	0	1,066
« *Any dog* »											0		0
ERIG	15	0	14	0	201	1	2,330	0	0	2	2,560	3	2,563
No ERIG	16	0	8	0	53	0	165	0	0	0	242	0	242
Total	31	0	22	0	254	1	2,495	0	0	2	2,802	3	2,805

* Two rabies-attributed deaths that occurred before the patients could have received the 4th session or not (early deaths. Abbreviations: ERIG: Equine rabies immunoglobulin.

Table 4: Rabies deaths observed by intradermal post-exposure prophylaxis completion allocation hypotheses for the two early deaths, Fisher mid-point p value per hypothesis, probability of occurrence of each hypothesis, and weighed Fisher mid-point p value, among patients bitten by a confirmed rabid dog, 2003-2014, Institut Pasteur du Cambodge.

PEP completion hypothesis	Observed	Hypothesis 1		Hypothesis 2		Hypothesis 3	
		Both early deaths would not have completed the full 4+ protocol		*One of the two early deaths would have completed the full 4+ protocol*		*Both early deaths would have completed the full 4+ protocol*	
Number of sessions completed		3	4+	3	4+	3	4+
N rabies deaths	3	3	0	2	1	1	2
N survived	1,717	126	1,591	126	1,591	126	1,591
Total	1,720	129	1,591	128	1,592	127	1,593
Unilateral Fisher mid-point value		0.00020642		0.0080455		0.1105589	
Probability of occurrence of hypothesis°		0.0054646		0.1369171		0.8576183	
Weighed overall unilateral Fisher mid-point value for all 3 hypotheses		0.0959					

° Among only those receiving 3 or 4+ sessions and omitting the two early rabies deaths to allocate.
Note: The individual probabilities derived from the logistic regression model for the two "early deaths" to be allocated to the 3-sessions group were 7.23% and 7.34%, respectively, leading to an overall weighed Fisher p value of 0.0961, very near to the initial estimate of 0.0959.

Table 5: Distribution of selected socio-demographic and baseline clinical characteristics of non-immune patients residing outside Phnom Penh, who completed 3 sessions vs. 4+ sessions of Vero cell-based rabies intradermal post-exposure prophylaxis and not lost to follow-up, after a bite by a confirmed rabid dog only, 2003-2014, Institut Pasteur du Cambodge.

	Patients who received		
	3 intradermal PEP sessions only* (n=127)	4 or 5 intradermal PEP sessions (n=1,591)	p value**
Age (years) – median (IQR)	19 (11-35)	17 (9-38)	0.544
<15 years old	52 (40.94%)	703 (44.18%)	0.720
15-65 years old	71 (55.90%)	826 (51.92%)	0.386
>65 years old	7 (5.51%)	141 (8.86%)	0.195
Male	75 (59.06%)	911 (57.26%)	0.694
Bite category (12)			
Category II	90 (70.87%)	1,112 (69.89%)	0.818
Category III	37 (29.13%)	479 (30.11%)	
Anatomical site°			
Foot/leg	75 (59.06%)	901 (56.67%)	0.601
Hand	38 (29.92%)	501 (31.51%)	0.711
Head / neck	9 (7.09%)	125 (7.86%)	0.754
Other bite characteristics			
Number of bite wounds	2 (2-2)	2 (2-2)	0.749
One bite wound	13 (10.24%)	114 (7.17%)	0.203
Two bite wounds	91 (71.65%)	1,210 (76.05%)	0.266
Three bite wounds or more	23 (18.11%)	267 (16.78%)	0.700
Documented suture	0 / 27 (0.00%)	13 / 345 (3.77%)	0.612◇
Clothes interposed	31 (24.41%)	409 (25.71%)	0.833
Documented wound care	35/52 (67.31%)	444/695 (63.88%)	0.548◇
Dog status			
Spontaneous bite	111 (87.40%)	1,337 (84.04%)	0.375
Sick-looking	119 (93.70%)	1,520 (95.54%)	0.374
Identified owner	117 (92.13%)	1,327 (83.41%)	**0.008**
N persons bitten	2 (1-3)	2 (1-3)	0.864
One person only	59 (46.46%)	778 (48.90%)	0.596
Two persons	35 (27.56%)	375 (23.57%)	0.310
More than two persons	33 (25.98%)	438 (27.53%)	0.707
Spontaneous death documented▽	6 (4.72%)	121 (7.61%)	0.232
PEP characteristics			
Year of PEP– median (IQR)	2009 (2007-2012)	2010 (2008-2012)	0.273
Delay before PEP (days)	1 (0-2)	1 (0-1)	0.125
Same day (Day 0)	35 (27.56%)	454 (28.54%)	0.814
After 1-6 days	91 (70.54%)	1,125 (70.71%)	0.968
After one week (>Day 6)	2 (1.57%)	12 (0.75%)	0.322
ERIG received	120 (94.49%)	1,546 (97.17%)	0.090
Follow-up			
Delay until callback (months) – median (IQR)	27.8 (6.6-51.3)	23.8 (6.6-47.1)	0.210
≥ 12 months	78 (61.42%)	910 (57.23%)	0.359

* Excluding 2 patients who died before they could have received the 4th session or not; **Fisher, Chi-square or Wilcoxon p-value (p-values <0.05 shown in bold); ° Non-exclusive categories as multiple bites on various anatomical sites are possible;◇ These variables were poorly documented. ▽ The majority of biting dogs were immediately put down, before they could die spontaneously; Abbreviations: IQR: interquartile range; PEP: post-exposure prophylaxis; ERIG: Equine rabies immunoglobulin.

Table 6: Description of the association between rabies death and selected socio-demographic and clinical characteristics of non-immune patients residing outside Phnom Penh who received more than 2 sessions of Vero cell-based rabies intradermal post-exposure prophylaxis and not lost to follow-up after a bite by a confirmed rabid dog only, 2003-2014, Institut Pasteur du Cambodge.

Variable	Category	Rabies death	N alive	% death	p Fisher	Unadjusted Odds-ratio		p*
						Estimate	95% CI	
Age	Continuous	-	-	-	-	0.97	0.90 – 1.05	0.507
Age (years)	<15	2	754	0.26%	0.636	NE	-	-
	15-65	1	902	0.11%				
	>65	0	61	0.0%				
	<15	2	754	0.26%	0.586	2.55	0.23-28.22	0.444
	≥15	1	963	0.10%		Ref		
Sex	Male	3	985	0.30%	0.266	NE	-	-
	Female	0	732	0.0%				
Type of bite								
Bite category (12)	Category II	0	1,202	0.0%	0.027	NE	-	-
	Category III	3	515	0.58%				
Anatomical site of principal bite**	Bite to the Foot/leg	0	976	0.0%	0.081	NE	-	-
	No bite to Foot/leg	3	740	0.40%				
	Bite to the hand	1	539	0.18%	1.000	1.09	0.99-12.07	0.943
	No bite to the hand	2	1,177	0.17%		Ref		
	Bite to head/neck	2	133	1.51%	**0.017**	23.80	2.14-264.24	**0.010**
	No bite to head/neck	1	1,583	0.06%		Ref		
Other bite characteristics								
Number of bite wounds	Continuous	-	-	-	-	1.18	0.87-1.61	0.288
	One bite wound	0	127	0.0%	0.148	NE	-	-
	Two bite wounds	1	1,301	0.08%				
	≥ three bite wounds	2	289	0.69%				
	1-2 bite wound	1	1,428	0.07%	0.076	Ref	-	-
	> 2 bite wounds	2	289	0.69%		9.88	0.89-109.35	0.062
Documented suture◇	Suture	1/1	13/371	-	0.037◇	NE	-	-
	No suture	0/1	358/746	-				
Clothes interposed	Clothes interposed	0	440	0.0%	0.575	NE	-	
	No clothes interposed	3	1,277	0.23%				
Documented wound care◇	Wound care	1/1	479/480	-	1.000◇	NE	-	-
	No wound care	0/0	267/267	-				
Dog status								
Spontaneous bite	Spontaneous bite	2	1,447	0.14%	0.402	Ref		
	Provoked bite	1	270	0.37%		2.68	0.24-29.65	0.422
Sick-looking	Sick-looking dog	3	1,638	0.21%	1.000	NE	-	-
	Non sick-looking dog	0	79	0.0%				
Identified owner	Identified owner	3	1,443	0.21%	1.000	NE	-	-
	No identified owner	0	274	0.0%				
N persons bitten	Continuous	-	1,720	-	-	0.50	0.09-2.62	0.410
	One person only	2	836	0.24%	0.613	NE	-	-
	Two persons	1	410	0.24%				
	≥ two persons	0	471	0.0%				
	One person only	2	836	0.24%	0.615	2.11	0.19-23.29	0.543
	Two persons or more	1	881	0.11%		Ref		
Dog outcome at first PEP	Dog died spontaneously	0	127	0.0%	1.000	NE	-	-
	Dog alive, put down or disappeared	3	1,590	0.19%				
PEP characteristics								
Delay before PEP (days)	Continuous	-	-	-		0.25	0.03-2.16	0.208
	Same day (Day 0)	2	488	0.41%	0.219	NE	-	-
	Between 1-6 days	1	1,215	0.08%				
	≥ 7 days	0	14	0.0%				
	Same day	2	488	0.41%	0.197	5.04	0.46-55.67	0.187
	One day or more	1	1,229	0.08%		Ref		
3 or 4 sessions	3 sessions	1	126	0.79%	0.206	6.25	0.57-71.43	0.134
	4 sessions or more	2	1,591	0.12%		Ref		
ERIG	ERIG received	3	1,665	0.18%	1.000	NE	-	-
	No ERIG	0	52	0.0%				

* Likelihood ratio test p (p-values <0.05 shown in bold); ** Non-exclusive categories as multiple bites on various anatomical sites are possible; ◇ These variables were poorly documented. Abbreviations: PEP: post-exposure prophylaxis; ERIG: Equine rabies immunoglobulin; NE: Not estimable/not convergent; CI: 95% confidence interval; Ref: Reference category with OR value of 1.

Supplementary Table ST1: Distribution of selected baseline socio-demographic and clinical characteristics of patients who received Vero cell-based intradermal rabies post-exposure prophylaxis after a bite by a confirmed rabid or a sick but untested dog ("any dog"), stratified by place of residence (Phnom Penh vs. other provinces)

	Phnom Penh (n=520)	Other Provinces (n=3,318)	p*
Age (years) – median (IQR)	24 (14-41)	19 (10-40)	**0.0003**
<15 years old	130 (25.00%)	1,310 (39.48%)	**<0.001**
15-65 years old	370 (71.15%)	1,878 (56.60%)	**<0.001**
>65 years old	20 (7.85%)	130 (3.92%)	0.102
Male	305 (58.65%)	1,874 (56.48%)	0.352
Bite category (12)			
Category II	446 (85.77%)	2,499 (75.32%)	**<0.001**
Category III	74 (14.23%)	819 (24.68%)	
Anatomical site of the principal bite**			
Foot/leg	183 (35.19%)	1,880 (56.67%)	**<0.001**
Hand	307 (59.04%)	1,066 (32.13%)	**<0.001**
Head / neck	26 (5.00%)	233 (7.02%)	0.087
Other bite characteristics			
Number of bite wounds – median (IQR)	2 (1-2)	2 (2-2)	**<0.001**
One bite wound	141 (27.12%)	320 (9.64%)	**<0.001**
Two bite wounds	329 (63.27%)	2,526 (76.13%)	**<0.001**
Three bite wounds or more	50 (9.61%)	472 (14.22%)	**0.004**
Documented suture◇	4/132 (3.03%)	28/1,048 (2.67%)	0.775◇
Clothes interposed	96 (18.46%)	819 (24.68%)	**0.002**
Documented wound care◇	70/167 (41.92%)	810/1,591 (50.91%)	**0.028◇**
Dog status			
Spontaneous bite	215 (41.35%)	2,634 (79.39%)	**<0.001**
Sick-looking	511 (98.27%)	3,227 (97.26%)	0.178
Dogs tested positive	129 (24.81%)	1,461 (44.03%)	**<0.001**
Identified owner	473 (90.96%)	2,772 (83.54%)	<0.001
N persons bitten – median (IQR)	1 (1-2)	1 (1-3)	**0.015**
One person only	311 (59.81%)	1,755 (52.89%)	**0.003**
Two persons	90 (17.31%)	692 (20.86%)	**0.006**
More than two persons	119 (22.88%)	871 (26.25%)	0.103
Spontaneous death documented°	151 (29.04%)	321 (9.67%)	**<0.001**
PEP characteristics			
Year of PEP– median (IQR)	2007 (2005 – 2013)	2011 (2008 – 2013)	**<0.001**
Delay before PEP (days) – median (IQR)	1 (1-3)	1 (1-2)	**0.012**
Same day (Day 0)	109 (20.96%)	703 (21.19%)	0.907
After 1-6 days	372 (71.54%)	2,504 (75.47%)	0.057
After one week (>Day 6)	39 (7.50%)	111 (3.34%)	**<0.001**
ERIG received	274 (52.69%)	2,931 (88.34%)	**<0.001**
N PEP sessions– median (IQR)	4 (3-4)	4 (4-5)	**<0.001**
1-2 sessions	18 (3.46%)	69 (2.08%)	0.057
3 sessions	186 (35.77%)	336 (10.13%)	**<0.001**
4+ sessions	306 (58.84%)	2,913 (87.80%)	**<0.001**

2003-2014, Institut Pasteur du Cambodge.

* Chi-square or Wilcoxon test (p-values <0.05 shown in bold); ** Non-exclusive categories as multiple bites on various anatomical sites are possible; ° The majority of biting dogs were immediately put down, before they could die spontaneously: ◇ These variables were poorly documented. Abbreviations: IQR: interquartile range; PEP: post-exposure prophylaxis; ERIG: Equine rabies immunoglobulin.

Note: Among Phnom Penh residents compared to patients included in the study, age tended to be higher, bites tended to be single and less severe, more often to the hand but less to the head/neck, biting dogs bit less spontaneously, and dogs' owners were more often identified. The number of persons bitten by a given dog was lower and dogs were more often observed as dying spontaneously (but usually put down immediately in the countryside). Bites occurred less often through cloth but wound cleansing was less well documented. ERIG was less often prescribed and the number of PEP sessions was lower. Phnom Penh residents had been managed significantly longer ago, as the attendance by residents of other provinces grew across the years.

Supplementary Table ST2: Distribution of selected baseline socio-demographic and clinical characteristics of patients who received Vero cell-based intradermal rabies post-exposure prophylaxis after a bite by a confirmed rabid or by a sick but untested dog ("any dog"), stratified by follow-up status (included patients vs. lost to follow-up) 2003-2014, Institut Pasteur du Cambodge.

	Patients included (n=2,805)	Patients lost to follow-up (n=513)	p*
Age (years) – median (IQR)	20 (10 – 40)	19.5 (10 – 37)	0.530
<15 years old	402 (14.33%)	63 (12.28%)	0.219
15-65 years old	2,380 (84.85%)	449 (87.52%)	0.116
>65 years old	23 (0.82%)	1 (0.19%)	0.124
Male	1,575 (56.15%)	299 (58.28%)	0.370
Bite category (12)			
Category II	2,088 (74.44%)	411 (80.12%)	**0.006**
Category III	717 (25.56%)	102 (19.88%)	
Anatomical site of the principal bite**			
Foot/leg	1,613 (57.52%)	267 (52.05%)	**0.021**
Hand	880 (31.38%)	186 (36.26%)	**0.030**
Head / neck	195 (6.95%)	38 (7.41%)	0.712
Other bite characteristics			
Number of bite wounds – median (IQR)	2 (2 – 2)	2 (2 – 2)	**0.016**
One bite wound	250 (8.91%)	70 (13.65%)	**0.001**
Two bite wounds	2,151 (76.68%)	375 (73.10%)	0.080
Three bite wounds or more	404 (14.40%)	68 (13.25%)	0.494
Documented suture◇	26/921 (2.82%)	2/127 (1.57%)	**0.005**
Clothes interposed	707 (25.20%)	112 (21.83%)	0.103
Documented wound care◇	717/1,404 (51.07%)	93/187 (49.73%)	0.756
Dog status			
Spontaneous bite	2,279 (81.25%)	355 (69.20%)	**<0.001**
Sick-looking	2,722 (97.04%)	505 (98.44%)	0.074
Dogs tested rabies-positive	1,739 (62.00%)	118 (23.00%)	**<0.001**
Identified owner	2,343 (83.53%)	429 (83.63%)	0.957
N persons bitten – median (IQR)	1 (1 – 3)	1 (1 – 3)	0.282
One person only	1,469 (52.37%)	286 (55.75%)	0.158
Two persons	596 (21.25%)	96 (18.71%)	0.194
More than two persons	740 (26.38%)	135 (26.32%)	0.975
Spontaneous death documented°	243 (8.66%)	78 (15.20%)	**<0.001**
PEP characteristics			
Year of PEP– median (IQR)	2011 (2008 – 2013)	2007 (2005 – 2013)	**<0.001**
Delay before PEP (days) – median (IQR)	1 (1 – 2)	2 (1 – 3)	**<0.001**
Same day (Day 0)	630 (22.46%)	73 (14.23%)	**<0.001**
After 1-6 days	2,107 (75.12%)	397 (77.39%)	0.298
After one week (>Day 6)	68 (2.42%)	43 (8.38%)	**<0.001**
ERIG received	2,563 (91.37%)	368 (71.73%)	**<0.001**
N PEP sessions – median (IQR)	4 (4 – 5)	4 (4 – 5)	**0.022**
1-2 sessions	53 (1.89%)	16 (3.12%)	0.137
3 sessions	257 (9.16%)	79 (15.40%)	**<0.001**
4+ sessions	2,495 (88.95%)	418 (81.48%)	**<0.001**
Delay before callback			
Delay (months) – median (IQR)	26.93 (9.7-46.87)	46.47 (29.42-97.68)	**<0.001**
≥ 12 months	2,062 (73.54%)	494 (96.48%)	**<0.001**

*Fisher, Chi-Square or Wilcoxon (p-values <0.05 shown in bold); ** Non-exclusive categories as multiple bites on various anatomical sites are possible; ° The majority of biting dogs were immediately put down, before they could die spontaneously; ◇ These variables were poorly documented. Abbreviations: IQR: interquartile range; PEP: post-exposure prophylaxis; ERIG: Equine rabies immunoglobulin.

Note: Among patients lost to follow-up compared to patients included in the study, bites tended to be less severe, less often to the lower limbs and more often to the hand. The number of bite wounds was lower and biting dogs bit less often spontaneously; Dogs were somewhat more often sick-looking but bit a lower number of persons. These dogs died more often spontaneously but were less often put down after the bite. The delay in PEP was longer in patients lost to follow-up, they received ERIG less often and received fewer rabies vaccine doses, but patients lost to follow-up had been managed significantly longer ago.

Table ST3: Rabies deaths observed by intradermal post-exposure prophylaxis completion allocation hypotheses for the two early deaths, Fisher mid-point p value per hypothesis, probability of occurrence of each hypothesis, and weighed Fisher mid-point p value, among patients bitten by confirmed rabid and sick but untested dogs ("any dog"), 2003-2014, Institut Pasteur du Cambodge.

PEP completion hypothesis	Observed	Hypothesis 1		Hypothesis 2 °		Hypothesis 3	
		Both early deaths would not have completed the full 4+ protocol		*One of the two early deaths would have completed the full 4+ protocol*		*Both early deaths would have completed the full 4+ protocol*	
Number of sessions completed		3	4+	3	4+	3	4+
N rabies deaths	3	3	0	2	1	1	2
N survived	2,749	254	2,495	254	2,495	254	2,495
Total	2,752	257	2,495	256	2,496	255	2,497
Unilateral Fisher test mid-point value for each hypothesis		0.0003986		0.0124483		0.1381057	
Probability of occurrence of hypothesis°		0.0085983		0.1682578		0.8231438	
Weighed overall unilateral Fisher mid-point value for all 3 hypotheses		0.1158					

° Among those receiving 3 or 4+ sessions and omitting the two early rabies deaths to allocate

Note: The probability for patients to have referred for 3 sessions was 9.59%. The probability that the two early deaths would have both received 3 sessions (Hypothesis 1), that one or the other would have received 4+ sessions (Hypothesis 2) or that both would have received 4+ sessions (Hypothesis 3) were therefore 0.86%, 16.83% and 82.31%, respectively. The sum of the mid-p values for Hypotheses 1-3, each weighed by their likelihood, yielded an overall unilateral Fisher mid-point estimate of 0.1158 (non-significant) when comparing deaths among patients who received 3 or 4+ sessions after being bitten by a confirmed rabid dog (Table 4).

The individual probabilities derived from the logistic regression model for the two "early deaths" to be allocated to the 3-sessions group were 0.0658 and 0.0689, respectively, leading to an overall weighed Fisher p value of 0.1325, a little higher than the initial estimate of 0.1158.

Table ST4: Distribution of selected socio-demographic and baseline clinical characteristics of non-immune patients residing outside Phnom Penh, who completed 3 sessions vs. 4+ sessions of Vero cell-based rabies intradermal post-exposure prophylaxis and not lost to follow-up, after a bite by a confirmed rabid or sick but untested dog ("any dog"), 2003-2014, Institut Pasteur du Cambodge.

	Patients who received		p°
	3 intradermal PEP sessions only* (n=255)	4 or 5 intradermal PEP sessions* (n=2,495)	
Age (years) – median (IQR)	26 (12-39)	19 (10-40)	0.067
<15 years old	65 (25.49%)	1,012 (40.56%)	**<0.001**
15-65 years old	181 (70.98 %)	1,379 (55.27%)	**<0.001**
>65 years old	9 (3.53%)	104 (4.17%)	0.624
Male	141 (55.29%)	1,398 (56.03%)	0.821
Bite category (12)			
Category II	195 (76.47%)	1,847 (74.03%)	0.395
Category III	60 (23.53%)	648 (25.97%)	
Anatomical site of the principal bite**			
Foot/leg	154 (60.39%)	1,426 (57.18%)	0.236
Hand	82 (32.16%)	779 (31.23%)	0.762
Head / neck	13 (5.10%)	179 (7.18%)	0.215
Other bite characteristics			
Number of bite wounds – median (IQR)	2 (2-2)	2 (2-2)	0.452
One bite wound	22 (8.63%)	219 (8.78%)	0.935
Two bite wounds	202 (79.22%)	1,909 (76.51%)	0.330
Three bite wounds or more	31 (12.16%)	367 (14.71%)	0.270
Documented suture◇	3/69 (1.17%)	23/832 (0.92%)	0.443◇
Clothes interposed	57 (22.35%)	638 (25.57%)	0.260
Documented wound care◇	66/111 (25.49%)	645/1,272 (25.85%)	0.092◇
Dog status			
Spontaneous bite	203 (79.61%)	2,036 (81.60%)	0.435
Sick-looking	246 (96.47%)	2,424 (97.15%)	0.536
Identified owner	226 (88.63%)	2,069 (82.93%)	**0.020**
N persons bitten – median (IQR)	1 (1-3)	1 (1-3)	0.929
One person only	134 (52.55%)	1,304 (52.26%)	0.931
Two persons	52 (20.39%)	531 (21.28%)	0.740
More than two persons	69 (27.06%)	660 (26.45%)	0.835
Spontaneous death documented	29 (11.37%)	210 (8.42%)	0.110
PEP characteristics			
Year of PEP– median (IQR)	2011 (2008-2013)	2011 (2008-2013)	**0.033**
Delay before PEP (days) – median (IQR)	1 (1-3)	1 (1-2)	**<0.001**
Same day (Day 0)	49 (19.22%)	576 (23.09%)	0.160
After 1-6 days	193 (75.69%)	1,867 (74.83%)	0.764
After one week (>Day 6)	13 (5.10%)	52 (2.08%)	**0.002**
ERIG received	202 (79.22%)	2,330 (93.39%)	**<0.001**
Follow-up			
Delay until callback (months) – median (IQR)	36.6 (16.9-62.5)	26.1 (9.0-45.77)	**<0.001**
≥ 12 months	206 (80.78%)	1,809 (72.53%)	**0.005**

* Excluding 2 patients who died before they could have received the 4th session or not; ° Fisher, Chi-square or Wilcoxon test p values (p-values <0.05 shown in bold); ** Non-exclusive categories as multiple bites on various anatomical sites are possible ◇ These variables were poorly documented. Abbreviations: IQR: interquartile range; PEP: post-exposure prophylaxis; ERIG: Equine rabies immunoglobulin.
The unadjusted odds ratio of the association between rabies death and receiving three PEP sessions only was estimated at 4.44 [95% CI: 0.15 - 58.47] for patients bitten by "*any dog*".

Supplementary table ST5: Description of the association between rabies death and selected socio-demographic and clinical characteristics of non-immune patients residing outside Phnom Penh who received more than 2 sessions of Vero cell-based rabies intradermal post-exposure prophylaxis and not lost to follow-up after a bite by a confirmed rabid or an untested, sick-looking dog ("any dog"), 2003-2014, Institut Pasteur du Cambodge.

Variable	Category	Rabies death	N alive	% death	p Fisher	Unadjusted Odds-ratio		p**
						Estimate	95% CI*	
Age (years)	<15	2	1,096	0.18%	0.620	NE		
	15-65	1	1,549	0.06%				
	>65	0	104	0.0%				
	<15	2	1,096	0.18%	0.567	3.01	0.27-33.31	0.368
	≥15	1	1,653	0.06%		Ref	-	-
Sex	Male	3	1,538	0.19%	0.261	NE		
	Female	0	1,211	0.0%				
Type of bite								
Bite category (12)	Category II	3	707	0.42%	**0.017**	NE		
	Category III	0	2,042	0.0%				
Anatomical site of principal bite°	Bite to the Foot/leg	0	1,580	0.0%	0.077	NE		
	No bite to Foot/leg	3	1,168	0.26%				
	Bite to the hand	1	861	0.12%	1.000	1.09	0.10-12.10	0.940
	No bite to the hand	2	1,887	0.11%		Ref	-	-
	Bite to head/neck	2	191	1.04%	**0.014**	26.77	2.42-296.61	**0.007**
	No bite to head/neck	1	2,557	0.04%		Ref	-	-
Other bite characteristics								
Number of bite wounds	One bite wound	0	241	0.0%	0.078	NE		
	Two bite wounds	1	2,111	0.05%				
	≥ three bite wounds	2	397	0.50%				
	1-2 bite wound	1	2,352	0.04%	0.057	Ref	-	-
	> 2 bite wounds	2	397	0.50%		11.85	1.07-130.98	**0.044**
Documented suture◇	Suture	1	25	4.00%	**0.029**	NE		
	No suture	0	875	0.00%				
Clothes interposed	Clothes interposed	0	695	0.0%	0.576	NE		
	No clothes interposed	3	2,054	0.15%				
Documented wound care◇	Wound care	1	710	0.14%	1.000	NE		
	No wound care	0	672	0.0%				
Dog status								
Spontaneous bite	Spontaneous bite	2	2,238	0.09%	0.461	Ref	-	-
	Provoked bite	1	511	0.20%		2.19	0.20-24.19	0.523
Sick-looking	Sick-looking dog	3	2,669	0.11%	1.000	NE		
	Non sick-looking dog	0	80	0.0%				
Identified owner	Identified owner	3	2,294	0.13%	1.000	NE		
	No identified owner	0	455	0.0%				
N persons bitten	One person only	2	1,437	0.14%	0.606	NE		
	Two persons	1	583	0.17%				
	> Two persons	0	729	0.0%				
	One person only	1	1,312	0.08%	1.000	Ref	0.17-20.0	0.623
	Two persons or more	2	1,437	0.14%		1.82	-	-
Dog outcome at first PEP	Dog died spontaneously	3	2,510	0.12%	1.000	NE		
	Dog alive, put down or disappeared	0	239	0.0%				
PEP characteristics								
Delay before PEP (days)	Same day (Day 0)	2	624	0.32%	0.197	NE		
	Between 1-6 days	1	2,060	0.05%				
	≥ 7 days	0	65	0.0%				
	Same day	2	624	0.32%	0.132	6.81	0.62-75.24	0.117
	One day or more	1	2,125	0.05%		Ref	-	-
3 or 4 sessions	3 sessions	1	254	0.39%	0.253	4.93	0.44-50.0	0.194
	4 sessions or more	2	2,495	0.08%		Ref	-	-
ERIG	ERIG received	3	2,531	0.12%	1.000	NE		
	No ERIG	0	218	0.0%				

* CI: confidence interval; ** Likelihood ratio test p (p-values <0.05 shown in bold); ° Non-exclusive categories as multiple bites on various anatomical sites are possible; ◇ These variables were poorly documented. Abbreviations: PEP: post-exposure prophylaxis; ERIG: Equine rabies immunoglobulin; NE: Not estimable/not convergent; Ref: Reference category with OR value of 1.

Part 5: Discussion, outcomes and perspectives

9. Discussion

9.1 Rabies eradication or human dog-mediated rabies control?

The vast majority of human rabies cases in Southeast Asia and Worldwide are caused by bites from non-immunized dogs[18,297,360]. Human rabies was prevalent in now-developed countries. It became neglected in the mid-20th Century after countries in Western Europe or North America, Israel, or Japan and Korea controlled the disease in humans through registration and vaccination of domestic animals[1].

Considering the vast number of dogs worldwide and the wildlife reservoir of rabies, eradication is out of the question[361]. Rabies control in dogs is feasible but requires vaccine coverage to be maintained: Rabies was rolled back in the Malayan peninsula in the 1950s[103], but has re-emerged. South America has now largely controlled dog-mediated rabies. But dogs and wild terrestrial fauna or bats will remain a reservoir and rabies will re-emerge unless dog and cat vaccination efforts are constantly maintained at least at 70%[103,362–366]. Considering the lifetime expectancy of dogs in the developing setting[367] and annual costs of vaccination in a high-turnover and large dog population such as in Cambodia[67–70], achieving even this is an ambitious goal.

Other than tourists traveling to endemic settings who pay for rabies vaccination, vaccinating populations before they are exposed to rabies is deemed too costly for already strained public health systems of developing countries. The exception could perhaps be small, isolated populations faced with an extremely high incidence of exposure to rabies[113,114], such as isolated population subgroups in the Amazon, for example[368].

The World Health Organization (WHO), the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) are currently spearheading an effort to eliminate dog-transmitted rabies worldwide by 2030[98,99]. This is aligned with Goal 3 of the United Nations Sustainable Development Goals[369].

While we strive for all dogs to be vaccinated, a major effort is urgently needed to make the time-proven and well-tolerated rabies vaccine (and immunoglobulin when indicated) post-exposure prophylaxis (PEP) geographically and financially accessible in a timely way to those people who remain the most vulnerable to rabies: The rural populations of developing countries[71,111].

9.2 Improving geographical accessibility of timely and adequate PEP

Abraham Wald observed damage to returning wartime bombers and compared his observations to expected damage predicted through modelling, in order to guide corrective measures[370]. It is in the same spirit that we based our analyses on PEP noncompletion and its estimated burden in Cambodian Districts as a proxy for obstacles to access[111].

Our study identifies distance as a factor independently associated with PEP noncompletion. But we chose a measure of *impact* rather than more approximate markers such as noncompletion itself, overall population, distance, etc. Had we chosen distance as the sole indicator, we would have set up a peripheral center in a district of a far-flung Cambodian province, therefore with a small population. Had we chosen district population as the sole indicator, we would have set up a center in Kandal province, with a very high population but neighboring Phnom Penh. Developing a model based on several factors and estimating the number of noncompleted PEP, we approach the strategy delineated by Jeffrey Rose and attempt to prioritize areas where the impact of building a peripheral center will be greatest[371].

The representativeness of our findings in Cambodia, however, may have changed over time. The road network progressively improved, becoming more extensive and of better quality. With a rising economy, Cambodians' income increased. The proportion of the population whose livelihood depends directly on harvesting paddy – a factor related to PEP noncompletion in our study - is also evolving. To allay these concerns, we studied only recent years (2009-2013) in our analysis. A report from the World Bank shows that the poverty rate fell before 2009 but that it receded more slowly between 2009 and 2013[372]. Between 2009 and 2012, the percentage of rural population changed little, going from 80.2% to 79.9% of the country total and the agricultural labor force going from 66.3% to 65% of the total labor force in Cambodia[373].

Can our strategy be extrapolated to other countries? Thailand, Vietnam and China has health centers capable of delivering PEP in cities of rural areas. But the PEP coldspot identification strategy can easily be used in countries with an equally rural population, equally dependent on annual harvests in Southeast Asia (Lao PDR, parts of Indonesia, China…), in South Asia and in Africa, parts of the World which bear the heaviest rabies burden[18].

The analytical strategy is applicable to any disease as long as: 1/ Its prevalence is not too high (<10%); 2/ The background incidence can be estimated in the population; 3/ There is a single, near-exhaustive database or recruiting center. In the absence of a single database, the strategy can be applied by grouping databases. This requires service delivery centers to be exhaustively included. This also requires concatenable data, collected using the same questionnaires, definitions and electronic data-entry

forms. Centralized identifiers can be used for patient management during successive referrals and to assess double entry. A card bearing the identifier number can be delivered to the patient who would have to continue treatment elsewhere, preventing double entries across centers. A capture-recapture method can also be used to estimate the overlap in center databases.

Setting up peripheral rabies PEP centers requires investment and great caution to ensure competence and sustainability. Staff can be employed, trained and monitored by Institut Pasteur du Cambodge (IPC), a research institute of the Ministry of Health of Cambodia. All centers must use the same questionnaires, standard operating procedures, protocols, procedures and injection devices best suited to intradermal injection. Administrative and financial management should also be ensured by IPC.

The issue remains of whether these peripheral vaccine PEP centers should also deliver RIG. Indeed, RIG are in short supply worldwide, extremely costly[71] with a short shelf life and require strict cold chain procedures. RIG access in peripheral centers could be improved by cost—reduction strategies. Fragmenting RIG doses by limiting RIG use to only local infiltration of the wound may help reduce costs[303]. Further dilution of doses may be another RIG dose-sparing option[374] to help provide those peripheral centers with access to RIG.

Beyond these options, patients could be triaged using point-of-care testing methods for biting dogs once these have been humanely euthanized. But these still require skillful brain extraction or tissue sampling[59].

Another approach could be to use a triage algorithm on-site based on dog syndromic evaluation. Peripheral centers which administer PEP only would thereby refer the patient to the capital for rabies testing to be performed on the dog head and RIG to be administered if necessary.

9.3 Improving financial accessibility of timely and adequate PEP

Our study found no difference in percentages of rabies deaths among patients who received a full, 4+ sessions PEP regimen compared to noncompleters who received an abridged, one-week, three-session PEP regimen.

The statistical power of our study, however, was limited. This is to be expected, as WHO-recommended PEP regimens and prequalified vaccines are highly effective[336]. Comparative studies on PEP regimen effectiveness are confronted with this as well as several other issues.

Comparing PEP regimens could rely on randomized clinical trials (RCTs). These are widely considered to bring the strongest proof of efficacy and effectiveness[375]. It is critical that all clinical trials undertaken adhere to good clinical practice (GCP) including ethical approval and informed consent and never against placebo in rabies PEP trials[376]. RCTs randomly allocate patients to the established or new intervention group, optimally blinding patients and clinicians to treatment assignment. From a

methodological standpoint, patients and clinicians can be blinded to the vaccine being used in rabies PEP RCTs but not blinded to the regimen trialed if the number of doses or volume is different, unless a placebo dose is injected. More importantly, RCTs may not be the best solution to provide first evidence of the noninferiority of new rabies vaccines or regimens, for ethical reasons: trialing an abridged rabies PEP regimen without solid prior evidence would be unethical if one recommended regimen is already effective against a highly lethal disease[377,378]. The proposed new regimen must have an established PEP comparator vaccine and regimen[376]. Whether any new regimen evaluation should include RIG for all patients or RIG only as available in the country remains a hotly debated ethical question[379,380].

Based on our experience, we therefore argue that PEP regimen abridgment should first be based on careful assessment of observational data, if necessary as part of a clinical network using standardized questionnaires and procedures, at best subsequently confirmed by a RCT.

An observational study suffers from patients not being randomized, with the difference in percentage of rabies deaths being potentially explained by factors other than PEP sessions received. Several other issues may arise when using observational data to assess clinical effectiveness of a new regimen, especially if the sample size can be achieved only through a collaborative network. As discussed in our paper on clinical outcome, RIG infiltration may eliminate the rabies virus inoculum *in situ*, thereby overestimating the effectiveness of an abridged rabies PEP regimen. The rabies PEP regimens recommended by WHO to March 2018 are extremely effective. The number of rabies deaths despite timely and adequate PEP – the clinical endpoint - is in the order of 1-2 p. 1,000 even in rabies-exposed patients in our study. New techniques using different markers – for example detection of *in situ* RNA replication or other biomarkers at the wound site – may in the future reduce the need for clinical endpoints.

Nerve-tissue vaccines are much cheaper than modern cell-based vaccines but associated with a high risk of side effects. The WHO has recommended the discontinuation of NTV use (and production) since 1983[61,128]. These are still produced in Algeria, Bolivia and Ethiopia and existing stocks are being used in a few other countries. The rabies vaccines currently in use are based on cell cultures. Marketed vaccines must all meet the WHO criteria, including antigenicity (≥2.5 IU per IM dose as determined by the mouse protection potency test) and have to be shown noninferior to the reference human diploid cell vaccine[135]. To 16/08/2017, a total of 17 cell-based vaccines were currently produced worldwide (of which 9 in China), among which only two are WHO-prequalified (L. Knopf, personal communication). Even if the cold chain is respected, the effectiveness of rabies vaccines may vary. Regimens should therefore be compared using WHO-prequalified vaccines which were used to establish the recommended protocols.

In the case of intradermal regimens, staff must be properly trained to measure and administer the vaccine dose and to properly infiltrate all wounds with RIG, after diluting the RIG dose if necessary. Virological testing of the biting dog's head is not easily accessible everywhere[59]. Pasteur Institutes are among the very few worldwide where PEP, virological testing and data analysis teams are all located in a single site. In other settings, a strong collaborative network associating these teams must be established locally to conduct PEP studies.

Evaluating or introducing a new regimen or vaccine is an opportunity to implement a pharmacovigilance system. This system must call back all PEP patients exposed to rabid or highly suspect dogs after 6 or if possible 12 months. A verbal autopsy method can be used to investigate reported deaths and attribute these to rabies or to another cause, preferably with an external review board. Any probable rabies death despite PEP must be notified to health authorities and to the manufacturer. Each PEP failure must be an opportunity to examine events and improve procedures, if needed using a root cause analysis and producing an Ishikawa "fishbone" diagram[381].

10. Outcomes and perspectives

10.1 Outcomes

Following the conclusions of our mapping study published in the article on PEP coldspot identification[111], the Institut Pasteur in Cambodia obtained from Cambodian health authorities the authorization to create a peripheral IPC/MOH rabies clinic in Battambang to serve populations in the western part of the country. This is in line with the Cambodian health authorities' national implementation of the WHO strategy to eliminate dog-mediated human rabies by 2030[356–359].

In July 2016 the author was selected to participate to a 2016-2017 WHO working group of the Strategic Advisory Group of Experts (SAGE) on rabies composed of 10 international rabies experts, a SAGE chair and a World Health Organization secretariat[382]. Preliminary results of the IPC clinical study were shared with the working group during a 20-22 June 2017 meeting in Geneva. This working group endorsed the reduction of rabies PEP to an IPC regimen of three sessions of two intradermal 0.1 mL doses of rabies vaccine over a period of one week. This was again presented by the author as spokesperson for the working group at the general SAGE meeting in Geneva on October 18, 2017 which endorsed the working group's recommended change in PEP regimen. The WHO received the final SAGE report in December 2017 and endorsed this new regimen in its 2018 recommendations published in the Weekly Epidemiological Record dated April 20, 2018[113]. The now-recommended "IPC regimen" is the

first one-week rabies PEP regimen ever to be endorsed by WHO. As with a 2006-2009 study on HIV[383] this is a second instance of the data collected by IPC - for Cambodia and from Cambodia - changing clinical management recommendations worldwide.

Additionally, knowledge translation was a priority during the time that this PhD was written. These efforts and outcomes are detailed in Annex Chapter 7.

10.2 Perspectives

GAVI, the Vaccine Alliance, has over the past decade been hesitant to financially support rabies PEP due to its complexity and the time required for a full PEP course. It did, however, decide in 2016 to support a WHO-led research agenda on rabies and PEP. There is good reason to believe that the WHO-recommended abridgment of PEP from a one-month to a one-week IPC protocol will enable GAVI to finally support PEP in 47 GAVI-eligible countries in its next Vaccine Investment Strategy[115].

The callback system implemented at IPC for the clinical lookback study has now become routine. This will provide much-needed pharmacovigilance data to verify the safety and further support the IPC rabies PEP regimen. This system is recommended in the article and should help encourage others to do the same, to the benefit of their patients as well as for potential sharing of information at the international level.

The conclusions of the RESIST 0/1 clinical study presented as part of this PhD are supported by preliminary data of an ongoing, RESIST-2 study on immune response and rabies-neutralizing antibody titers before and two weeks after the fourth injection in patients bitten by rabies-confirmed dogs. This study is being completed, the manuscript is in preparation and will soon be submitted for publication.

The RESIST-2 study on short-term immune protection will be complemented by a RESIST-3 study on long-term antibody protection. This study will trace back IPC patients who received a full PEP regimen or who abridged it to three sessions of their own accord two, five, or 10 years earlier. Pre-booster antibodies will be titered to compare long-term protection between a then-full and an abridged PEP regimen, using an antibody titer $\geq$0.5 UI/mL as a proxy for protection.

A RESIST-4 study is ongoing with a Paris team specialized in health economics. Using patient attendance data at the IPC rabies prevention clinic following the introduction of cost participation and each successive raise, this study will examine the acceptability and quantitative impact on rabies clinic attendance of one US$ increase in patients' participation to rabies vaccination costs.

A RESIST-5 study was undertaken to examine the probability of dog head positivity for rabies, in view of dog behavior and bite and other characteristics. The resulting algorithm will be essential for patient triage in peripheral rabies clinics when deciding to refer to the capital for rabies immunoglobulin. This study, conducted by B. Mollo and suggested and supervised by the author was the topic of a master

degree in epidemiology. A dissertation was written and received high marks. Findings were communicated at several international conferences[66] and a manuscript is in preparation.

A submitted paper on the clinical evaluation of vaccines makes the case for a standardized patient evaluation form to guide PEP and document outcome. If such a form were to be officially endorsed by WHO, outcome in non-completers could be rapidly and effectively documented worldwide thanks to web-based data exchange networks. This would help detect regimen or vaccine lot failures, or allow for adequately-powered studies to be rapidly set up, at a modest cost. Standardizing the rabies PEP center toolbox across the IPIN would be a very constructive and useful first step. This is being discussed.

In addition to numerous studies on rabies conducted by the Institut Pasteur (Paris) team throughout the years, the RESIST studies (including those published as part of this PhD) will help reposition the Pasteur Institute and the Pasteur Institute International Network on the frontline of rabies prevention and care. To this author's knowledge, some Institutes of the Network are the only sites worldwide where vaccination teams administering PEP, virology teams performing rabies diagnosis in dog heads and epidemiology teams capable of clinically following up patients and analyzing data are all integrated and located on the same campus, usually in close and longtime collaboration with reference hospitals. This opens exciting perspectives for future research on issues such as subcutaneous administration of rabies PEP, further regimen abridgment to two PEP sessions, development and testing of new vaccines, changes in the use of rabies immunoglobulin or - perhaps one day - the use of antivirals in the preventive or curative management of human rabies.

After millennia of powerlessness, success in this last endeavor would finally begin to close the last chapter in humankind's lethal confrontation with rabies.

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Annex

1. Four Thousand Years of Concepts Relating to Rabies in Animals and Humans, its Prevention and its Cure

Note: This is an excerpt from a more extensive published, open-access review of the historical literature: Tarantola A. Four Thousand Years of Concepts Relating to Rabies in Animals and Humans, Its Prevention and Its Cure. Trop Med Infect Dis. 2017 Jun; 2(2): 5 available at https://www.mdpi.com/2414-6366/2/2/5

Rabies is an ancient and much-feared disease. Over the centuries, many different authors—clinicians, veterinarians, surgeons, pharmacists but also writers, philosophers, and poets—have mentioned rabies in their writings. These have been published in greater detail by the author in a recent open-access review[1].

1.1 Pasteur and his time

Around the turn of the 19th Century, the scientific approach improved the understanding of the physiopathology and clinical epidemiology of rabies, which was remarkably summarized by Samuel Cooper in 1823[205].

1.1.1 Understanding the transmission of rabies

Much experimental work was done on the transmission of rabies[121,384–386] - and its prevention through the amputation (Helman, cited in[38]) or immunization of animals[38,72,121,387]. In 1804 in Jena (in present-day Germany), Georg Zinke transmitted rabies experimentally (without a bite) by applying the saliva of rabid dogs to animals' tissues[72,121,384,386,388,389]. The same was demonstrated in 1813 by Hugo Altgraf zu Salm-Reifferscheidt[78] and prior to 1814 by François Magendie and Gilbert Breschet, this time using saliva from a human rabies patient[390–392]. In 1805 in Turin, Francesco Rossi reported having experimentally transmitted rabies to dogs by inserting sciatic nerve segments of rabid cats into a fresh wound[393]. Clinicians progressively identified the seat of rabies infection in the midbrain[72,121] and nerve ending density was positively correlated with risks of infection and migration[38,394].

1.1.2 Understanding the origin of rabies

In the struggle pitting the microbial theory against "spontaneous generation", subsequent experiments provided solid scientific evidence to support the long-suspected transmission of rabies by "filterable" infectious agents present in the saliva[121,384,387]: Magendie in 1842 suspected that the agent was not an inert poison but a "virus" capable of multiplying and developing in the host[395,396]. Magendie then Casimir Davaine in 1872 experimented on the increase in virulence by serial passage (but these were with septicemia and anthrax bacteria, not with viruses)[397–399]. In 1880, Edmond Nocard succeeded in separating saliva into two components, one non-infective and the other infective[400]. The agent of rabies was now considered to progressively ascend from the infected wound to the brain not through the blood but through the nerves - as initially hypothesized in 1879 but not established by Paul-Henri Duboué[401] - before diffusing centrifugally[38,121,389,395].

1.1.3 Preventing rabies transmission

Resorting to nerve section as a means of preventing rabies had been contemplated by George Hicks in 1807[402]. Duboué - who communicated his findings to L. Pasteur on January 12,1881[400] - also postulated that the rabies "virus" could be destroyed *in situ* or prevented from reaching the *medulla oblongata*[394]. This paved the way for the advent of post-exposure prophylaxis, based on the notion of taking advantage of the weeks-long incubation period and rapidly building the patient's immunity through timely and adequate vaccination[121].

Putting John Hunter's recommendations into practice, Eusebio Valli, an Italian physician, claimed to have carried out experimental infections and successfully immunized dogs by injecting the saliva of rabid dogs after submitting it to gastric juices of frogs in 1799. He claimed to have inoculated this mixture to at least two people in Pisa bitten by a suspected rabid dog and who did not contract rabies[403–405]. This author was unable to access original sources, but Apollinaire Bouchardat, a pharmacist of the Veterinary Faculty in Lyons, France, is cited as having postulated in the 1850s that dogs could be immunized against rabies as a public health measure[214]. In 1879, at the Veterinary school also in Lyons, rabies pioneer Pierre-Victor Galtier inoculated rabies to a rabbit through cutaneous injection, administered rabid dog saliva intravenously to a sheep which did not contract rabies after challenge but became immunized, theorized post-exposure prophylaxis and began experimenting on vaccination of dogs[406,121,407–415]. Henry Toussaint - another veterinarian - conducted research in Lyons on heat- and subsequently carbolic acid-attenuated anthrax vaccine in 1880[415,416]. Paul Gibier from the Faculty of Medicine and the Muséum d'Histoire Naturelle de Paris, showed in 1883-1884 that the rabies virus lost virulence after desiccation and that this approach could be used in humans[39,417].

1.1.4 The Pasteur team and experimental research on rabies

It is in this already extremely rich and advanced research context that Louis Pasteur and his colleagues at the École Normale Supérieure in Paris began to apply their systematic, rigorous and data-driven scientific methods to the study of rabies in December of 1880[3,39,401]. Pasteur and his team had already developed an effective attenuated fowl cholera vaccine[418], were working on an attenuated anthrax vaccine and strove to apply their techniques to rabies - a much-feared and highly symbolic disease, albeit known to be controllable by veterinary measures alone[400,419]. An experimental model of rabies was developed by Paul Emilio "Emile" Roux* in dogs inoculated after trepanation, and later in the noticeably more manageable rabbit[3,121]. A "fixed", adapted, rabies virus strain of "exalted virulence" with shorter incubation times and unfailing transmission could then be selected through successive passage in the rabbit, paving the way for a reliable experimental model and methodical approach. After discussing it in 1881[422], Pasteur and his team endeavored in 1882 to develop a canine "vaccine" (thus named in honor of Edward Jenner), using after 1884 the desiccation technique also developed by Emile Roux to attenuate this live, highly virulent virus[2,120,122,423]. Rabies virus attenuation was first validated by experiments which Pasteur and his team reported in 1884, documenting survival of dogs vaccinated by live, attenuated vaccine before viral challenge.

1.1.5 Prophylaxis against rabies

The prototypal vaccine against rabies was first used as salvage therapy in humans presenting signs of declared clinical rabies, with rapid documented failure in at least one instance: that of the child Antoinette Poughon in late June 1885[3,424]. The vaccine, however, was to meet resounding success in patients exposed to rabies virus but with yet no signs of declared rabies.

History remembers a 9-year old schoolboy, Joseph Meister (Figure 5), attacked and bitten 14 times by a dog while on an errand in Maisonsgoutte (Meissengott), in then German-occupied Alsace, on July 4, 1885[2]. This episode is reviewed in detail in two newly accessible detailed publications[1,425] (and now accessible on the IPC website†). Jacques-Joseph Grancher administered subcutaneously the first doses of live attenuated rabies vaccine on July 6, 1885, at

* According to published sources, Emile Roux was motivated to work on rabies due to a personal experience. Then aged 11 and born in Confolens, he had been marked by dramatic events which occurred at Villemalet, in nearby La Rochette commune, Charente: Around midday on October 5, 1874, a rabid wolf had attacked Marie Favraud and Firmin Fontroubade, both aged 10, mauling and killing Marie Favraud on the spot. Two adults (Jean Texier, 31, and Jean Fontroubade, 28) came to the rescue, killed the wolf with their bare hands but were exposed. Jean Texier was bitten 40 times, with 25 severe wounds and Jean Fontroubade had an injury ("section") to a little finger. Twenty-eight days later, on November 1st, Jean Fontroubade developed signs of rabies and was helped to die three days after that, leaving a pregnant wife. Jean Texier presented signs of rabies on November 8 and also was helped to die, leaving a wife and two children. Fundraising activities were conducted to assist their families financially. Ten-year old Firmin Fontroubade was apparently unbitten and survived [420,421] (personal communication Mr. Vincent Ringeade, Mayor of La Rochette, October 2017).

† Accessible at http://www.webcitation.org/6oOwdJq7l

8:00 PM in the presence of Louis Pasteur - who, as a chemist, was not authorized to perform injections - and Alfred Vulpian. The first injection was derived from the chord of an inoculated rabbit which had died of rabies on 21st June (15 days earlier)[3]. Over the 10 following days, Joseph Meister received 12 additional doses of attenuated and progressively more virulent virus to quickly generate an immune response, in an attempt to beat the virus in a deadly race against time[38,426,427]. Meister survived.

Figure 5: Joseph Meister in 1885, the first human to have received Pasteur's live, attenuated rabies vaccine on July 6, 1885 as post-exposure prophylaxis (© Institut Pasteur - Musée Pasteur).

This successful attempt was repeated in late October 1885 in a second case, that of a 15 years-old shepherd, Jean-Baptiste Jupille from Villers-Farlay, Jura, who sustained on October 14 a deep bite to the left and right hands after an attack by a furious dog[2,3]. Jupille was referred to Pasteur and was the second to receive rabies post-exposure prophylaxis (PEP) in Paris from 20 to 30 October, 1885. Following Grancher's accidental exposure to the attenuated vaccine during Jupille's PEP, Adrien Loir and Eugène Viala became the two first humans to receive *pre*-exposure rabies vaccination[214].

For the very first time since its first recorded description 3,800 years earlier[1], and despite some failures due mostly to delayed referral[3,122], clinicians now had a proven and effective means of rabies prevention in humans. This led to Louis Pasteur's laboratory at École Normale Supérieure in Paris to routinely offer PEP services.

The rabbit chord used in the Pasteur vaccination protocol was known to preserve its virulence despite preservation in carbolic acid[2]. It was, however, not stabilized and therefore not usable outside Paris unless "transported" by/in inoculated rabbits. Patients therefore had to travel to access PEP, in some cases across continents or oceans[428,429]. After 16 of 19 Russian patients

survived who came for PEP to Paris from Smolensk after being attacked by a rabid wolf [430], Elie Metchnikoff was named Director of the first center in Odessa established specifically to produce rabies vaccine (which benefited from Louis Pasteur's support) and implemented the "Pasteur treatment" in June 1886[431–433]. The not-for-profit, non-governmental Institut Pasteur Foundation was incorporated in France by a decree on 4 June 1887. The Institut Pasteur itself was built and inaugurated on 14 November 1888, after an unprecedented national and international movement and fundraising campaign to further disseminate PEP and to pursue research[401,426,428].

1.2 Modern Developments

1.2.1 Perfecting and expanding the reach of the rabies vaccine

Over the decades that followed the development of PEP by Pasteur and his team, many rabies prevention centers or "Pasteur institutes" - some affiliated with the Institut Pasteur in Paris, most not[432] - appeared across the Old and the New World. In 1909 there were 75 such centers worldwide, including in then Indochina[38,434]. These centers cultured *in vivo* then attenuated highly virulent rabies virus (RABV) locally, bringing the vaccine to the patients rather than the converse. In Saigon, animal bite victims received PEP as early as 1891, becoming the first to receive rabies PEP in Asia, Africa or Latin America (Figure 6)[4,5,435–437]. This was facilitated by RABV preservation in glycerin, also developed by Emile Roux and Albert Calmette[401,438], which no longer required uninterrupted sequences of RABV inoculation to successive unfortunate rabbits every ten days to preserve live virus.

Post-exposure prophylaxis biologicals and procedures were improved in the ensuing decades. The rabies vaccine was further refined by Emile Roux[5,14], Victor Babes[38], Follen Cabot[439], Claudio Fermi, Endre Högyes[8] and especially David Semple[6–8].

Figure 6: Albert Calmette and the first two patients to receive rabies PEP in Asia (excluding the Russian Empire), Africa or Latin America, 18 April 1891. The handwritten legend indicates that these were Malay children referred from Singapore (© Institut Pasteur)

Semple's inactivated nerve tissue vaccine was developed using sheep brain tissue in 1911 at the Pasteur Institute in Kasauli, India. The vaccine had limited immunogenicity, could cause serious unwanted effects (albeit less so than Pasteur's live attenuated vaccine), required a tedious protocol and was painful (as experienced first-hand by the author in West Africa as a child in the 1970s). The League of Nations' health organization's bulletin reported 115,859 PEP recipients exposed to potentially rabid animals worldwide during 1932 - May 1934 among whom 439 (0.4%) were considered to have died of rabies[440]. The Fuenzalida nerve tissue vaccine, developed on the newborn mice, was affordable, better tolerated than Semple's vaccine. For decades it saved countless human lives, especially in the developing world [10].

After failed initial attempts at the Institut Pasteur in 1913[441], the rabies virus was successfully cultured *in vitro* through several passages in 1936[442]. In the 1960s, harvests of RABV grown in tissue cultures became increasingly pure[443] and normative methods were developed to standardize the potency of the various vaccines[9,133,389]. Vaccines were developed on myelin-free suckling mouse brains[10] or on duck or chicken embryos[444], until the advent of new, highly antigenic, better-tolerated cell-culture vaccines[125,427,445–447]. This allowed for the tedious Pasteur then Fuenzalida protocols to be progressively replaced by the shorter Essen and Zagreb protocols[238]. An oral vaccine was developed for wildlife in 1971[448]. Through canine population regulations and control rabies was eliminated from cities in the industrialized World and elsewhere, including Shanghai in 1949 and Malaya in the early 1950s[103,449].

1.2.2 Identifying the virus

Research on the rabies virus itself made rapid advances. In 1903, Adelchi Negri described the first RABV-neuron interaction and Lina Luzzani-Negri described the diagnostic value of Negri bodies in infection with "street" rabies virus[450–452]. The rabies virus itself was first observed by electron microscope in the early 1960s[453–455]. The molecules produced by RABV (transcriptional mapping) were described in 1978[456] and the viral genes which code for them were sequenced in their entirety in 1988[21]. Direct and indirect diagnostic methods were developed to reliably confirm infection and antibody protection[9,59,457].

1.2.3 Modern-day advances

These advances led to the validation of rabies vaccine effectiveness, of shorter and dose-sparing regimens and of the equivalence of the intradermal vaccination route[85,282,302]. It also enabled the identification of nonfatal cases of RABV infection in animals[458–461] and in humans[56,368,462]. A very few human survivors of clinical rabies were documented, mostly in the New World following bat exposure[463–469].

1.3 In total

Rabies became a neglected disease when it was eliminated from Europe and North America. It is emerging in some island territories and remains uncontrolled in most of the developing Old World, where surveillance of dog bites, rabies exposures (syndromic or laboratory-confirmed) or rabies deaths is poor[62,470]. The prevention of human rabies deaths in the 21st century still relies on tools and strategies developed in the 19th century: Effective primary prevention of animal bites and responsible dog ownership as delineated by Fleming (in 1872)[72]; Canine vaccination as proposed by H. Bouley (in 1884)[38] and timely and effective rabies post-exposure prophylaxis using vaccine developed by Roux then Pasteur and his team and first administered in 1885 and immunoglobulin first developed by Babes in 1891.

2. Virological aspects of rabies

Although the mainly dog-mediated, prototypical rabies virus (RABV) is the cause of the overwhelming majority of cases in animals and humans worldwide, rabies as a clinical syndrome can be due to several species of Lyssaviruses. As opposed to all other rhabdoviruses[471], lyssaviruses are not vector-borne and have become adapted for direct transmission[23]. As with all other warm-blooded animals, all species of the Orders *Carnivora* and *Chiroptera* may be affected by lyssaviruses, with jumps to new species driven by evolutionary biology but perhaps first and foremost because of ecological and population determinants[19,472].

2.1 Classification and phylogeny

2.1.1 Classification of Lyssaviruses

Order *Mononegavirales*, Family *Rhabdoviridae*, Genus *Lyssaviruses* includes 16 lyssaviruses (an additional 2 are being characterized) (ICTV, 14/05/2018). Among these lyssaviruses, the *Rabies lyssavirus* (RABV) transmitted by dogs presents the largest public health burden and is the prototypical virus. Lyssaviruses share with RABV a similar structure and bullet-shaped morphology (Figure 7). To date, seven have been associated with rapidly-progressive fatal encephalomyelitis (rabies) in humans.

Phylogenetic analyses are mostly based on genetic variability of the nucleoprotein (N) gene, not least because of its role in vaccine development and response[473,474]. RABV is relatively stable compared to other RNA viruses, leading to distinct and well-set lineages. This classification

allows for phylogenetic studies reflecting epidemic trends, differing animal reservoirs, dog movement and human activities over prolonged time periods[475–477].

2.1.2 Origin and reservoir

A recent analysis points to the spread of a common ancestor for current circulating dog-mediated RABV during the 16th and 19th Centuries[19]. This confirms and narrows earlier estimations[17,478,479]. To date, however, attempts have not determined the exact time and place where and when the very first rabies virus originated.

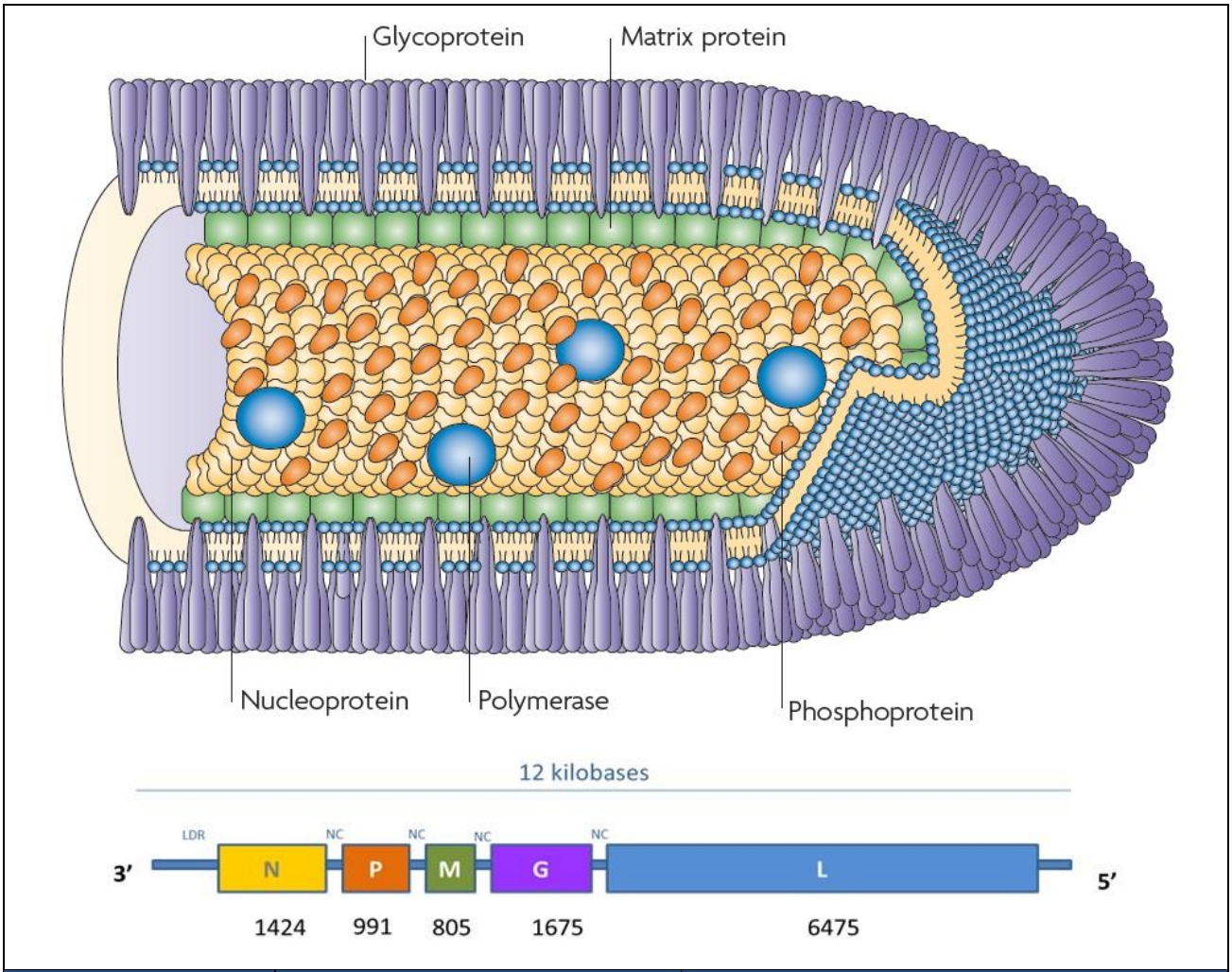
Bats seem to be "a" if not "the" reservoir for all lyssaviruses with the exception of Moloka and Ikoma viruses, for which the reservoir is not yet identified[16,23,171,480–485]. Phylogenetic analyses across lyssaviruses point to bats as the ancestral reservoir[17]. The relative immunity of bats – some of which present a host-pathogen response different from that of other non-flying carnivores - to rabies remains debated[16]; Their sheer numbers may be a major determinant in their role in maintaining virus circulation, especially in the Americas[19,483,486]. The vast majority of human rabies cases occurring worldwide, however, follow the bite of a rabid dog[18]. From a clinical epidemiology standpoint, dogs may therefore rightly be considered to be the reservoir for rabies transmitted to humans in Africa as in Asia, which bear the heaviest rabies burden[18,475,487–491].

2.2 Structure

The RABV genome is a 12,000 nucleotides-long, nonsegmented, single-stranded, negative-sense RNA genome. This RNA is transcribed into five mRNA segments coding for five viral proteins[21] (Figure 7). As the genome is negative-stranded, a positive-sense RNA - which subsequently acts as the matrix for the synthesis of a reverse and complementary strand of negative polarity - must be synthesized. The replication cycle is shown in Annex Chapter 2. The virus encodes its own RNA polymerase (the “Large” or “L” protein). The absence of proofreading mechanisms for the rabies virus RNA-polymerase is considered to be a strong driver for genetic diversity[492,493].

The G protein plays a major role in virus uptake by muscular or neuronal cells, cell-to-cell spread and pathogenicity[22]. The RABV virion attaches its G protein spikes to the cell surface receptors before entering the cell (see Annex Chapter 2).

Figure 7: Architecture and functional organization of the rabies virus (adapted from [23,25,494,495])



Protein	Role	Implications
nucleoprotein (N)	Encases RNA and is highly immunogenic	Targeted by specific antibodies for highly performant Direct Fluorescent Antibody Testing (DFAT)[59]
phosphoprotein (P)	Associated with ribonucleoprotein (RNP) core and stabilization of newly synthesized N	Potential therapeutics by affecting interaction of P protein and signal transducers and activators of transcription (STAT)[496]
matrix protein (M)	Associated with envelope and RNP; may be central to virus assembly and budding	Potential therapeutics by altering its role in the modulation of innate immunity[497]
glycoprotein (G)	Spikes on virus surface, binds to neuronal cells and facilitates axonal transport; Major antigen	Strongly immunogenic and used to develop an effective vaccine and neutralized by RIG. Potential recombinant rabies inactivated virus vaccine[498]
polymerase (L)	Associated with ribonucleoprotein core; replicates and transcribed viral RNA	Well conserved among lyssaviruses: can be used for diagnosis (PCR)[499]

Endocytosis is followed by the uncoating and release of the ribonucleoprotein complex (RNP)[23,24]. The virion polymerase transcribes the negative-sense RNA of the N, P, M, G and L genes into mRNA. These are then translated in the cell to produce the corresponding proteins while the RNA is replicated. In cells the accumulated viral proteins form intracytoplasmic inclusions which - when found in neuronal cells of the Ammon's Horn of the hippocampus - were termed Negri bodies and long considered pathognomonic of rabies[24,450–452]. The proteins and RNA are reassembled into new RABV particles which bud from the infected cell through the action of the M protein supported by the G protein[25]. The M protein then mediates the separation of budding progeny[25]. These new virions can then infect a nearby cell or a cell at a distance, during a process again involving the G protein spikes which is a major determinant of neuroinvasiveness as it binds to receptors on neuronal cells and can enhance centripetal axonal transport[23].

2.3 Other roles of RABV proteins

The G protein is strongly immunogenic (and used to develop rabies vaccines), as is the ribonucleoprotein complex (RNP), composed of the RNA encased in the N, P and L proteins[12,23,500]. Highly performant Direct Fluorescent Antibody Testing (DFAT, see Section 9) is based on antibodies directed against the ("N") nucleoprotein. Current research on the ("L") RNA polymerase, the M or the P genes and their inhibition may provide avenues for the treatment or prevention of rabies in humans.

3. Pathophysiology and anatomopathology

3.1 Local RABV replication at the inoculation site

The rabies virus (RABV) can be inoculated directly into a nerve or near a nerve ending, leading to an extremely short incubation period[37,42]. The virological transcription and translation cycle described in the previous section, however, is thought to generally multiply RABV in the muscle (myocytes, some fibrocytes[26]) and dermal tissue[27] at the inoculation site. This triggers a local and general reaction as the host attempts to control the virus, while the latter seeks to escape the immune response[501,502]. The physiological reaction to RABV infection involves microRNA production in the cell. MicroRNA are absent from neurons, in which RABV replicates well; In

some cases, a RABV variant escapes microRNA and other responses and begins to actively replicate in the muscle[28]. In addition to inoculum size, this may contribute to explain: 1/ the fact that not all confirmed rabid bites progress to transmission, infection and consequent death; and 2/ some protracted incubation periods. The RABV local replication, crossing and transport to the CNS requires time, providing a delay during which post-exposure prophylaxis (PEP) may be administered to great effect[121]. All warm-blooded animals (including fowl[503]) may acquire RABV. Host characteristics, however, make animals' susceptibility to experimental RABV infection - and subsequent capacity to transmit - vary greatly: raccoons and skunks are more susceptible to rabies than wolves or foxes which are more susceptible than dogs which are much more susceptible than opossums, and humans are more susceptible than rats[29,504]. Like fowl, rabid humans or rats do not transmit RABV.

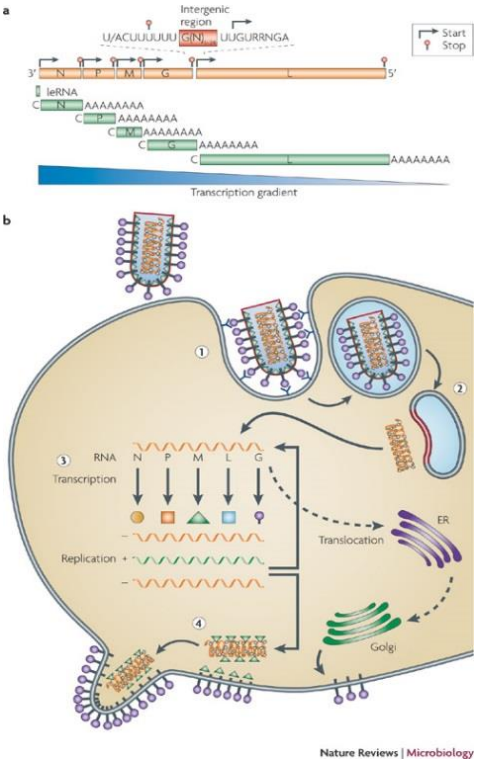
3.2 Transmission of RABV across the neuromuscular junction

It has been known early on that hematogenous spread of RABV is insignificant[121,30,38,39,2,505]. This is discussed in a recent publication by this author[32]. Similarly, possible sexual transmission of RABV is anecdotal and to this authors' knowledge has never been documented in humans[505,506]. RABV is a neurotropic virus: once it has begun to replicate in the muscle[26] (Figure 8), it crosses to peripheral nerves - motor and sensory[30,31,507] - close to the bite site[33]. It does so through the neuromuscular junction, at a site uncovered by the perineural sheath[30,31] (Figure 9).

3.1 (Centripetal) transport of RABV to the CNS

Once the neuromuscular junction has been forded, replication becomes more intense with peak titres of 10^5 pfu/mL supernatant in cultured neurons[504]. The virus does not replicate in axons, which are devoid of ribosomes. It proceeds within days through reverse axonal transport (periphery-to-CNS or centripetal migration) using microtubules and dynein towards the central nervous system (CNS): the spinal ganglia and the brain[33,34]. RABV is transported via the motor nerve to the ventral ganglia of the spinal cord, and perhaps in a delayed manner via the sensory nerves to the dorsal ganglia of the spinal cord[31,494]. Direct inoculation into a nerve is followed by centripetal ascension to the CNS where the neuronal cells are located[42,508] (Figure 10).

Figure 8: Schematic representation of the rabies virus replication cycle in an infected cell (from [494])



a | Transcription of the rabies virus genome. The encapsidated negative-stranded RNA (orange) serves as a template for transcription by the polymerase complex. Transcription starts with a short uncapped leader RNA (leRNA) from the 3' end of the genomic RNA. This is followed by the transcription of 5' end-capped (C) and polyadenylated (A) mRNAs, which encode the viral proteins (green). The polymerase complex stops at signal sequence (U/ACUUUUUU), ignores the intergenic region of 2–24 nucleotides and restarts transcription at the transcription start signal sequence (UUGURRNGA). Successful reinitiation of transcription at each gene junction does not always occur, therefore transcription is attenuated from the 3' to 5' end (this is illustrated by the transcription gradient).
b | A simplified rabies virus life cycle in an infected cell can be divided into three different phases. The first phase includes binding and entry into the host cell by endocytosis (step 1), followed by fusion of the viral membrane and endosome membrane to release the viral genome (uncoating; step 2). In the second phase, virion components are produced (transcription, replication and protein synthesis; step 3). The last phase of the life cycle is the assembly of the viral components and budding and release of the rabies virus virions (step 4), which can start a new round of infection. ER, endoplasmic reticulum.

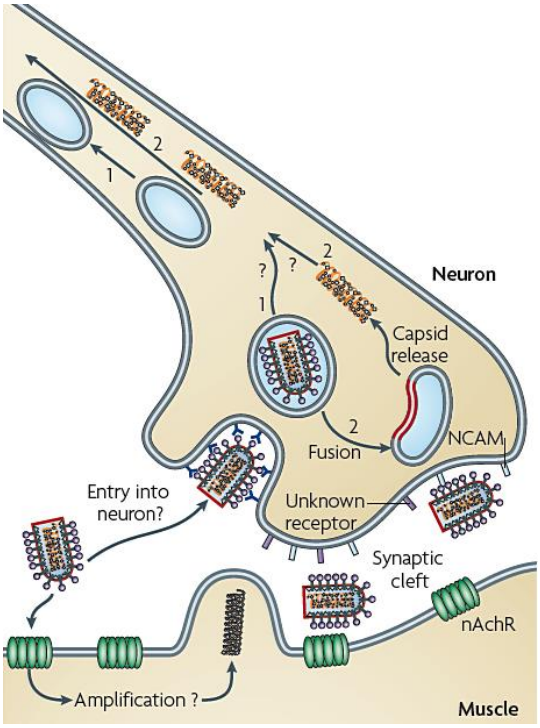


Figure 9: RABV passage across the neuromuscular junction and axonal transport (adapted from [494])

Experimental evidence from mainly *in vitro* and some *in vivo* studies suggest the following scenario.
After having replicated in the muscle cells at the inoculation site, RABV crosses the neuromuscular junction where the axon is unprotected [30].
Although nicotinic acetylcholine receptors (nACHr) are located on the muscle cell side of the neuromuscular junction, they are considered to play a role in transmission, perhaps by helping accumulate virus, especially at sites where the axon is unprotected [507]. The density of nACHr may vary greatly by animal species [29].
Neural cell adhesion molecule receptors (NCAMR) located in folds on the axonal side of the muscle-motor neuron junction may be a receptor for RABV [509]. The low-affinity p75 neurotrophin receptor is not present at the neuromuscular junction but may play a role in promoting RABV transfer between sensory neurons and the dorsal horn of the spinal cord [29,494,507].

The speed of the axonal transport in cultured sensory neurons has been estimated at 50-100 mm/day in experimental conditions[29,504]. A bite to the face or neck likely provides the virus with direct access to cranial rather than spinal nerves and therefore a speedier, more direct route to the brainstem, likely rendering PEP ineffective[31].

3.2 RABV replication in the CNS

Once in the spinal cord, the RABV virus replicates and progresses more quickly along anatomical pathways to the brainstem, where the virus has long been known to cause diffuse, nonspecific inflammation and signs of disease[29,50,72,394]. Negri bodies, long considered pathognomonic of rabies, are sites of viral replication but are not always present[510,511].

3.3 (Centrifugal) transport of RABV from the CNS

Once clinical (CNS) signs have appeared, RABV progresses very quickly via nerve pathways and through axon terminals to adjacent non-nervous tissue of peripheral organs, including salivary glands via the parasympathetic system[29,36]. Neurons in the adrenal medulla, cardiac ganglia and myocardium, skeletal muscle, plexuses of the gastrointestinal tract or even hair follicles may be affected[29]. Hair follicles are of particular interest, as they are used in diagnosis when sampled at the nape of the neck[47,61] (see Annex Chapter 5 on diagnostic techniques).

3.1 Viral excretion of RABV

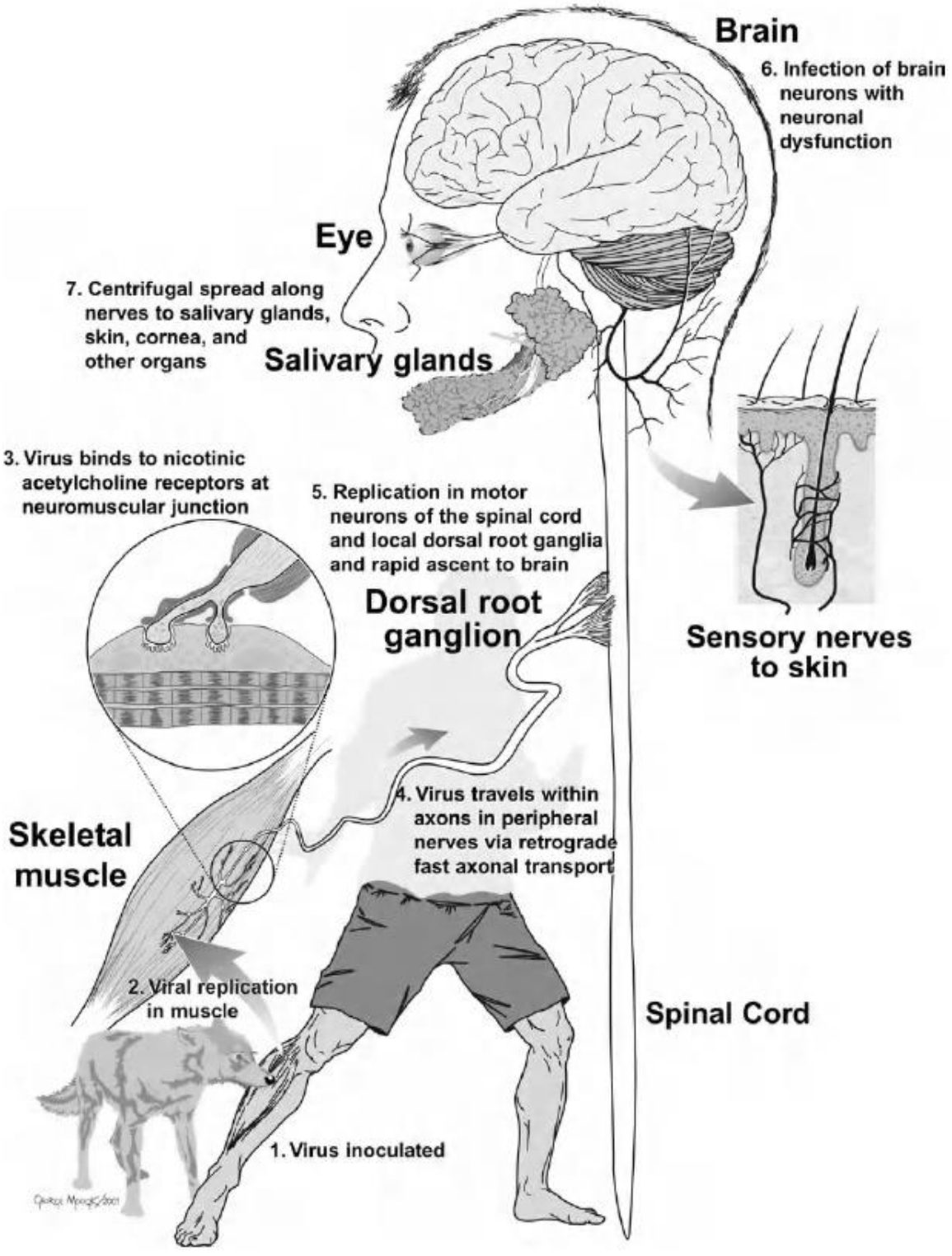
Rabies virus is present in the salivary glands and excreted in the saliva of infected dogs for a few days before the onset of clinical signs[36,512–514]. Some authors have hypothesized the role of excretion by the tongue, but this should not affect transmission risks or clinical and epidemiological considerations[31]. Rabies virus is shed intermittently in the saliva of rabid animals or humans after symptoms onset and until death[229]. RABV was not shed at all in the saliva of 52% rabid dogs in the experimental setting ([515] cited in [229]). Conversely, in very rare cases in India and Ethiopia, experimental dogs infected with wild, "street rabies" viral strains survived pauci-symptomatic rabies and excreted functional rabies virus for several months or years[29,458,516,517]. Rabies PCR in the CSF was described as remaining positive for more than four years in one single report on a human rabies survivor in India[518].

3.1 Neuronal impact of RABV infection

As noted as early as 1887, neuropathological findings following rabies infection are surprisingly tame[38]. Rabies is likely associated with neuronal dysfunction rather than observable neuronal destruction[35]. RABV does not seem to induce significant apoptosis in infected cells[494].

Available techniques, however, may not be performant enough to document neuronal dysfunction preceding cell death.

Figure 10: Schematic representation of the pathophysiological cycle of the rabies virus (RABV) after inoculation by the bite of a rabid dog (from [29])



3.2 Viral escape mechanisms

The mechanisms involved in RABV escaping the immune response in the replication site have been detailed by other authors[519] who aptly termed the rabies virus "subversive". The various escape mechanisms - including low-key replication and inhibition of immune response by viral N, P and M proteins - have been recently reviewed[31]. Complex and multiple, some of these mechanisms are not completely understood and are beyond the scope of the present review. Although experimentally attenuated rabies virus increases BBB permeability[520–523]. Faced with a mounting immune or cell-based response, "street rabies" virus induces apoptosis in T-cells but preserves the integrity of the blood-brain barrier. This further preventing antibodies to reach the virus, ultimately causing the subject's death.

3.3 RABV control mechanisms

3.3.1 Innate immunity

RABV infection with attenuated virus triggers the production of interferon-β and γ, chemokines (CCL-5, CXCL-10) and cytokines (IL-6 and IL-α, TNF-α) in the brains of experimental animals[29]. These reactions, however, are not observed experimentally with "street virus", which may deploy mechanisms to evade this response in the brain, likely *via* the viral phosphoprotein[31,520,521].

3.3.2 Cellular immunity

Release of chemokines and cytokines in the brain mobilize CD4 and CD19-positive lymphocytes, especially at the cerebellar level[29]. T cells also accumulate in infected brain tissue[521]. It is possible that in some instances RABV may be cleared in the CNS[521,524].

3.3.3 Humoral immunity

Before the virus crosses the neuromuscular junction to enter the neuron, the replication and diffusion of rabies viruses is hindered in the bite site by the humoral immune response targeted against the virus's G protein[520,521]. Pre-existing antibodies, acquired through pre-exposure immunization (veterinarians, astute travelers...) effectively prevents infection. Timely and effective prophylaxis after an exposure also is known since Roux and Pasteur's work to trigger an antibody response within a few days and prevent clinical rabies after a potentially infective bite[2,121]. The injection of equine or human rabies immunoglobulin at the inoculation site contributes to prevent replication and diffusion[11,185]. Rabies IgM or IgG antibodies, however, do not normally cross the blood-brain barrier (BBB) if it is intact and are of no use in clinically-declared rabies cases[521].

4. Clinical aspects of rabies

"I have seen agony in death only once, in a patient with rabies; he remained acutely aware of every stage in the process of his own disintegration over a twenty-four hour period, right up to his final moment."

Lewis Thomas, The lives of a cell: Notes of a biology watcher, 1974[525]

Rabies is an acute neurological syndrome caused by different lyssaviruses including RABV, which overwhelmingly leads to death in humans and in varying proportions in warm-blooded animals, especially terrestrial mammals and bats[526–528].

4.1 Risks and risk determinants of rabies transmission in humans following a bite by a RABV-infected animal

It has long been known that not all exposures to the saliva of rabid animals result in infection and clinical rabies in the bite victim[38,116,121,123,529,530]. In absence of timely and effective post-exposure prophylaxis (PEP), it has been reported that an estimated 20% of all victims bitten by a suspected but untested dog will develop rabies[18,353]. Historically, this has led to much confusion regarding supposedly effective "cures" for rabies.

4.1.1 Stratifying transmission data by dogs' rabid status

Data from the late 19th C mentions case-fatality rates after the bite of a suspected rabid dog of 15/145 (10.3%) in 1893 and 9/51 (17.6%) in 1894 in Nagasaki[531], 27/339 (19.0%) in then-Prussia (Faber cited in[38]), 44/470 (9.3%) in Hungary and 149/995 (15.0%) in 1870-1875 in an unspecified location, presumably Paris[38]. Much of these available historical data, however, pertains to bites by dog suspected but not tested for rabies.

Scarce sources provide information on outcome in people who did not receive PEP after being bitten by a confirmed rabid dog. A publication dated 1923 provided outcome data on 423 persons bitten by a suspected rabid dog in India who did not receive PEP, stating 148 deaths for a global 35.0% case-fatality rate[123]. Another publication mentions a 1946-1956 series of 541 persons bitten by confirmed rabid dogs in India, with 48.1% fatalities among 106 patients who did not receive PEP[265]. In a more recent and controversial 1984-1985 study in Thailand, nine (45.0%) of 20 persons who referred for deep wounds inflicted by confirmed rabid dogs and who refused nerve-tissue vaccine contracted rabies and died[296]. The aggregated data therefore points rather to

279 deaths (42.40%) among 658 persons bitten by rabid dogs who did not receive PEP (Tarantola A *et al.*, manuscript in preparation). The transmission rate despite PEP is discussed in Section 13.

4.1.2 Characteristics of the source

Host characteristics contribute to determine RABV viral load in saliva, perhaps explaining 13-16-fold higher documented transmission risks after bites from a rabid wolf compared to bites from a rabid dog[38,432,532].

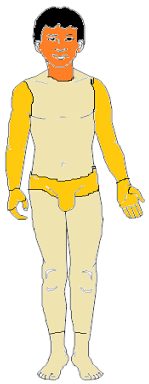
4.1.3 Types of exposure

Licks on nonintact skin are associated with lower transmission risks compared to bites, which determine wound management guidelines[132,533]. US recommendations consider scratches from rabid animals to entail risk, which has been said to be at least 50 times lower than after a bite although evidence is patchy at best[43,132]. Cases of human rabies, however, have been attributed to scratches only in India or Iran[27]and at least one human case after contact with a rabid dog's saliva (likely on nonintact skin)[534]. Furthermore, rare cases seem linked with butchering dogs or handling their fresh meat[535].

4.1.4 Anatomical site of the bite

It has long been known that anatomical sites, their degree of innervation and proximity to the brain are major determinants of RABV incubation and transmission risks[38,40,49,61,132,296,532,536,537]. Nerve density is a factor positively associated with transmission, but the exact role of nerve ending density and its attrition with age and sex is unclear[538,539]. Post-exposure prophylaxis failures and short incubation times may be due to direct inoculation of RABV into nerve endings[42,43]. Estimated probabilities of transmission after a bite from a rabid dog published by Babes, Sitthi-Amorn or summarized by Cleaveland *et al.*[38,40,296] are described by anatomical site in Figure 11.

Figure 11: Published estimated probability of rabies transmission following the bite of a rabid dog, by anatomical site.



Area	Babes [38]	Sitthi-Amorn[296]	Cleaveland [40]
Head/Neck	88%	100%	30-60%
Arm	30%	100%	15-40%
Hand	67%	100%	15-40%
Finger		NA	15-40%
Genitalia		NA	15-40%
Trunk	31%	NA	0-10%
Leg	21%	0%	0-10%
Foot	21%	0%	0-10%

Based on partial and syndromic data from Bangladesh, the compared risk of transmission from potentially rabid but untested dogs by anatomical site was as follows: Finger: 0%; Foot: 1%; Arm or leg: 2%; Hand: 3%; Trunk 4% and head/face/neck: 6%.[540]

4.2 Incubation period of (dog-mediated) rabies in humans

The time needed for RABV replication, passage into nerve and transport to the CNS to occur corresponds to the incubation period. This has long been studied[38,541] and the incubation period for rabies is said to be among the most variable of all diseases, ranging from a few days to several years[26] (up to eight years in a recently published case[542]). These outlying values, however, may largely be due to the fact that not all those exposed become infected after a given bite: Aside from direct inoculation into nerves which are thought to be associated with short incubation times, unrecognized earlier exposures to the virus may explain incubations (wrongly) classified as short, especially in endemic settings[29,543]. Conversely, others may die at a later date of a neurological illness wrongly labeled as rabies or due to unrecognized repeat exposure[544].

4.2.1 Rabies incubation in daily clinical practice

In daily clinical practice, clinical signs of rabies mostly (68%) appear between two weeks and three months following the infective bite (Table 8).

According to Babes *et al.*, the mean incubation period among 375 suspect rabies patients was 65-80 days and 46.5% of Brouardel's 447 cases occurred between Days 26 and 60 post-bite[38]. In a series of 110 cases in India, 61.8% of cases occurred between days 30 and 120 while 90.0% of cases occurred within 6 months following the presumed infective bite[545]. Among 104 cases in Bali, the incubation period ranged from 12 days to up to two years, and was less than 1 year in 98% of cases[46]. Documented incubation periods seemed comparable in a large series of 1,839 clinically suspect patients in the Philippines[49] while it was shorter in a series from Thailand[347].

In a smaller series of 49 rabies patients in the USA, the incubation period ranged 20 – 150 days with a mean incubation of 57.3 days[50]. Among 64 cases of rabies managed over a period of 15 years in a Beijing hospital, the median incubation time was 41 days with a mean of 69.7 days[535].

Table 8: Aggregated incubation data in 16 series of clinically suspected human rabies*.

		Reported incubation time				
		≤30 days	31-90 days	91-365 days	>365	Total
Matton 1867[505]	N	26	93	28	0	147
	%	*18.0%*	*63.0%*	*19.0%*	*0.0%*	*100.0%*
Comité d'Hygiène 1862-1872 (in [38])	N	38	129	23	0	190
	%	*20.0%*	*68.0%*	*12.0%*	*0.0%*	*100.0%*
Other Series (in [41])	N	464	846	226	19	1555
	%	*30.0%*	*54.0%*	*15.0%*	*1.0%*	*100.0%*
Other Series (in [38])	N	16	57	22	0	95
	%	*17.0%*	*60.0%*	*23.0%*	*0.0%*	*100.0%*
USA, 1960-1979[534] (for dog bites only)	N	3	6	1	1	11
	%	*27.3%*	*54.5%*	*9.1%*	*9.1%*	*100.0%*
Punjab, India, 1976-1983[545]	N	21	58	31	-	110
	%	*19.1%*	*52.7%*	*28.2%*	-	*100.0%*
Manila 1987-2006[49]	N	292	498	785	251	1826
	%	*16.0%*	*27.0%*	*43.0%*	*14.0%*	*100.0%*
Thailand (cited in [347])	N	509	120	28	49	706
	%	*72.0%*	*17.0%*	*4.0%*	*7.0%*	*100.0%*
Delhi, India 1998[546]	N	42	108	38	11	199
	%	*21.1%*	*54.3%*	*19.1%*	*5.5%*	*100.0%*
India, 8 communities, 2003-2004[547]	N	54	125	45	11	235
	%	*23.0%*	*53.2%*	*19.1%*	*4.7%*	*100.0%*
Bali** 2008-2010[46]	N	10	56	25	2	93
	%	*10.0%*	*60.0%*	*27.0%*	*2.0%*	*100.0%*
Abidjan, Côte d'Ivoire 2001-2009[548]	N	6	17	3	0	26
	%	*23.1%*	*65.4%*	*11.5%*	*0.0%*	*100.0%*
Rural Tanzania, 2002-2006[294]	N	12	12	2	0	26
	%	*46.1%*	*46.1%*	*7.7%*	*0.0%*	*100.0%*
Bangalore, India, 1980-2010[549]	N	10	3	9	3	25
	%	*40.0%*	*12.0%*	*36.0%*	*12.0%*	*100.0%*
Henan 2004-2009[550]	N	88	218	131	44	481
	%	*18.0%*	*45.0%*	*27.0%*	*9.0%*	*100.0%*
India, 2012-2014[551]	N	13	6	0	2	21
	%	*61.9%*	*28.6%*	*0.0%*	*9.5%*	*100 .0%*
Total	N	1,604	2,352	1,397	393	5,746
	%	*27.9%*	*40.9%*	*24.3%*	*6.8%*	*100.0%**

*Percentages may not add up to 100% due to rounding off; **Dr. M. Susilawathi, personal communication, 15/5/2015

4.2.2 Shortened rabies incubation

The duration of incubation is shorter in case of massive inoculation or of bites to highly innervated body parts such as the face: A bite to the face or neck likely provides the virus with

direct access to cranial rather than spinal nerves and a speedier, more direct route to the brainstem with a higher risk of transmission and likely shorter incubation[31,535]. In 139 observations, Babes cites a mean incubation period of 48 days following bites to the face, compared to 69 days for bites to the extremities[38]. A series of suspected cases from the Philippines also documented this difference in incubation times by anatomical site[49]. In Ghana, rabies following bites to the head in seven patients had an incubation of 16-46 days (average 31 days), while 44 cases of rabies following bites to the upper limb had incubation of 35-100 days (average 68 days)[347,552].

The incubation following atypical exposures such as organ transplantation, inhalation or butchering of rabid animals is unclear but limited available data point to incubation periods similar to those of rabies in bite victims[29,45,535,553].

4.3 Rabies in humans

There are an estimated 60,000 deaths from rabies worldwide each year[18] (five times the estimated fatalities from the 2013-2015 Ebola virus disease epidemic in West Africa[96]). These are overwhelmingly due to bites by non-immunized dogs[18,61,297,360,537,554–559]. Rabies presentation may vary somewhat in bat-transmitted rabies, which has been recently reviewed[31] but lies outside the focus of this document on dog-mediated rabies.

4.3.1 Prodromal phase

A few (2-7) days before the onset of symptoms, 30-70% of rabies patients report pain, paresthesia or pruritus at the bite site, perhaps reflecting involvement of the corresponding dorsal ganglia[44]. Fever is frequent, often high, but is by no means always present. In some cases, prodromes may mimic psychiatric symptoms, such as hallucinations, agitation or strangeness[549].

4.3.2 Clinical forms of human rabies

Following a prodromal phase, there are two main forms of canine-mediated rabies in humans: the paralytic form and the encephalitic form of rabies. This was long considered to depend to a large extent on which regions of the CNS are initially or principally affected[39,45]. In some cases of human rabies in Bali, the patients showed paralytic symptoms on admission and then developed encephalitic symptoms in later stages[560] (Table 9). Encephalitic rabies is followed by death within one week (usually 2-5 days) and paralytic rabies within two weeks, even with support therapy[37,43]. Survival was shown to be longer in paralytic forms in Thailand (11 days in paralytic rabies *vs.* 6 days in furious rabies) and in the USA[561,562].

Table 9: Clinical signs in 104 suspected human rabies cases, Bali, 11/2008-11/2010[46].

Furious/encephalitic rabies: 79.8%	Paralytic rabies: 20.2%
Hydrophobia (93.1%)	Urinary incontinence (27.5%)
Hypersalivation (88.2%)	Flaccid paralysis (21%)
Aerophobia (73.1%)	Abdominal discomfort (10.8%)
Dyspnea (74.5%)	
Photophobia (29.8%)	
Fever (18.2%)	
Convulsions (15.4%)	
Piloerection (4.8%)	
Muscle fasciculation (3.8%)	

4.3.2.1 Encephalitic rabies

This form is the classical form of the disease, and is also termed furious rabies (“rage furieuse”). It is reported to represent about 80% of infections in humans (70% in a series from Thailand[43]). The driving factor is the presence of neuronal dysfunction in the brainstem, cerebrum and limbic system - especially in the *cornu ammonis* of the Hippocampal formation of the temporal lobe[563–565] - clinically associated with periods of muscular and sensory excitability and arousal alternating with calmer periods during which the patient is terrified but awfully lucid. Hallucinations may be present, mimicking psychiatric illness[566]. Agitation or seizures are triggered by stimuli such as light or sounds or fanning air on skin ("aerophobia") or occurs spontaneously. Fever is inconstant, at less than 10% in one large series, and hypothalamic involvement may even lead to hypothermia[45,49]. Tapping muscles may lead to fasciculation. Pain is usually absent. Hydrophobia is classically considered synonymous with rabies [48] but - like aerophobia - is inconstant[47,49,50]. It is due to neuronal dysfunction of the spasm-inhibiting circuits of the brainstem which normally allows swallowing of liquid[567–569]. Stimulation of the pharynx by drink triggers uncontrollable reflex muscular spasms and gagging, which can lead to intense thirst, especially in febrile patients. Hydrophobia often enters into the case definition or is well-identified by clinicians; Its true incidence may therefore be overestimated in published case series[46,62]. When present, however, it is an extremely reliable indicator, especially in verbal autopsy studies[51,52]. Gastrointestinal bleeding may be present[45]. Neurological signs progress to spasmodic ataxic breathing, cardiac arrhythmia, coma and death. Magnetic resonance imaging may show moderate enhancement in hypothalamus, cerebrum, mammillary bodies, gray nuclei and cerebral lobes[565,570].

4.3.2.2 Paralytic rabies

Also called "dumb" rabies (“rage muette” or “rage tranquille” in old French-language reference material), this form is considered less frequent in humans[46,47]. The driving factor may

be dysfunction of peripheral nerves[561] or basal ganglia[45]. Although parasympathetic dysfunction may lead to hyperproduction of saliva, hydrophobia is much more rare in the paralytic form[45]. Striated and smooth muscle weakness is a major sign, ascending progressively. Altered sensorium and sphincter involvement are present and dysautonomia progresses to involvement of respiratory muscles and death. In rare cases, paralytic rabies may begin by other clinical forms, but without hydrophobia[321,571]. Magnetic resonance imaging may show enhancement in the spinal cord, medulla, brainstem and basal ganglia[565,570,572,573].

Paralytic rabies may be difficult to distinguish from Guillain-Barré syndrome[549,572]. Furthermore, many etiologies - such as cerebral malaria - may cause coma, febrile or otherwise in the tropical setting[53–55]. The consequences of the difficulty in clinically diagnosing paralytic rabies is that surveillance and detection of (predominantly furious) rabies systematically underestimates the rabies burden, especially in tropical countries with limited resources for diagnosis[18].

Whichever the inaugural clinical form, death occurs within a few days in all cases of human rabies, with only a few cases of documented survival, overwhelmingly with severe sequelae in case of dog-mediated rabies[56,549].

5. Rabies diagnostics

Note: This is a summary of a more extensive published, open-access review of the literature: Duong V, Tarantola A, Ong S, Mey C, Choeung R, Ly S, Bourhy H, Dussart P, Buchy P. Laboratory diagnostics in dog-mediated rabies: an overview of performance and a proposed strategy for various settings. Int J Infect Dis. 2016 May;46:107-14 available at https://www.ijidonline.com/article/S1201-9712(16)31003-7/fulltext

Dog-mediated rabies diagnosis in humans and animals has greatly benefited from technical advances in the laboratory setting. Approaches to diagnosis now include detection of rabies virus (RABV), of RABV RNA, of RABV antigens or of immune response to those antigens. We have recently published an in-depth review of these techniques and their use to meet various public health objectives[59].

Direct fluorescent antibody testing (DFAT) is the mainstay of rabies diagnosis in sampled tissue (*post-mortem* cerebral samples in biting animals or humans) worldwide[59]. Polyclonal antibodies targeting the RABV ribonucleocapsid or monoclonal antibodies targeting the RABV nucleoprotein (N) are made fluorescent using fluorescein isothiocyanate. Fluorescence is observed in microscopy in sampled tissues after lavage after 1-2 hours of contact. The sensitivity and specificity of DFAT nears 99% in experienced laboratories.

In rabies non-endemic settings or after rabies elimination laboratory diagnosis is imperative to guide public health measures, document protection (*intravitam* serology on vaccinees' blood samples) or to conduct research (seroprevalence studies, death despite timely PEP).

In resource-limited settings and/or rural areas of endemic countries, where most cases occur worldwide laboratory diagnosis is desirable but is extremely constrained. Public health surveillance needs in the endemic setting with high caseloads may therefore be satisfied by resorting to syndromic case definitions and past history of dog bite[51,62,294].

6. The burden of dog bites and dog-mediated rabies in Cambodia

6.1 Epidemiology of rabies in Cambodia

6.1.1 Epidemiological aspects in dogs

6.1.1.1 Number of dogs in Cambodia

In Cambodia, tradition, cultural beliefs and past insecurity[574] - compounded by dog husbandry and trade in some areas (including for butchering in Cambodia or Vietnam[553]) - have given rise to a huge canine population (Figure 12). Data gathered by the epidemiology team at Institut Pasteur du Cambodge during 2012-2013 community studies in Kampong Cham Province show that a total of 23,603 persons living in 4,203 homes owned a total of 4,645 dogs, among which 1,837 homes (44%) owned no dogs and 2,366 homes owned at least one dog (range 1-12 dogs; mean 2.0 ± 1.4; IQR) for a ratio of 19.7 dogs: 100 persons in total homes and 35.1 dogs: 100 persons in dog-owning homes (A. Tarantola *et al.*, IPC, unpublished). Earlier studies conducted by the Epidemiology & Public Health Unit at Institut Pasteur du Cambodge (epi@ipc) have found broadly similar dog-to-human ratios of approximately 1:3 in dog-owning homes of rural areas[67–70]. With 80% of the ~16 M Cambodian population being rural, this would translate into at least 4 M dogs in rural areas, which are overwhelmingly unvaccinated despite being owner dogs.

The human population of China is 100 times greater than Cambodia's, but its estimated dog population is only 32 times greater[557].

Figure 12: Two dogs (center) follow a Khmer convoy, Eastern Wall bas-relief, Prasat Bayon, Angkor, 12th-13th century AD, Cambodia (photo A. Tarantola).

6.1.2 Circulation of rabies in dogs in Cambodia

The only available data on rabies in dogs in Cambodia are provided by the national reference center for rabies diagnosis, virology unit, Institut du Pasteur Cambodge. These stem from biting animals' heads brought for testing by bite victims referred to the Rabies Vaccination Clinic at IPC. A total of 2,632 dogs' heads were brought for testing at IPC's reference laboratory from Jan 2000 to Dec 2014, inclusive. Among these, 1290 (49.0%) were positive, including 114/330 (34.5%) dogs' heads from Phnom Penh, the capital.

6.1.3 Dog bites in Cambodia

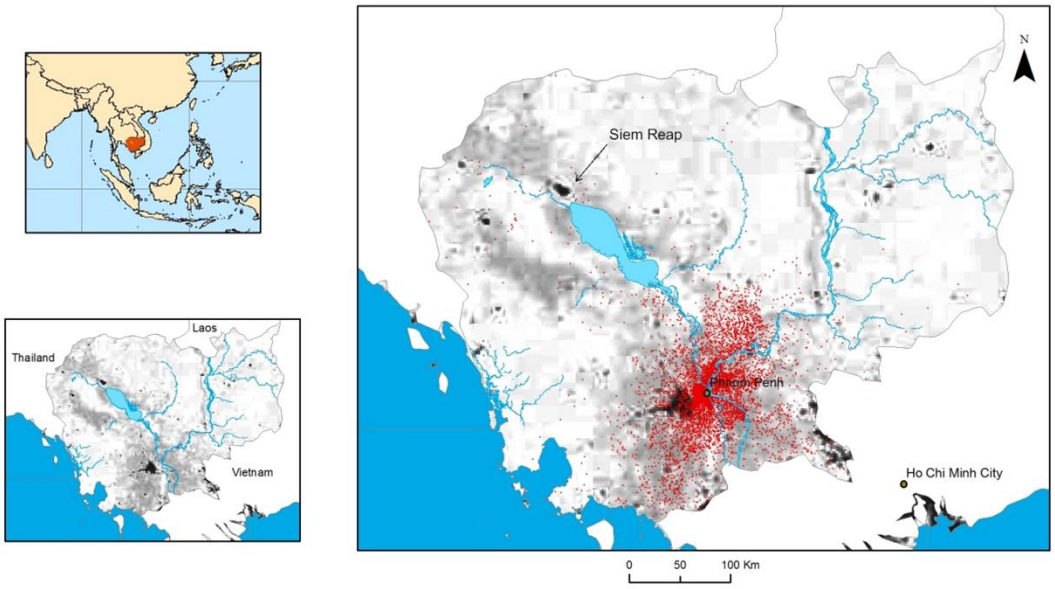
Data from a prospective study in rural villages of Siem Reap province, Cambodia, estimates the rate of bites by from unvaccinated dogs to be 40% higher than the highest previously published estimates worldwide, at 5 bites per 100 person-years (95% CI: 3.6-6.8)[67]. Using a different methodology, this was approximately the figure documented by the community studies in Kampong Cham province in 2012-2013 with an estimated incidence of 4 bites per 100-person-year, by the dog of another household in approximately 90% of cases (Epidemiology & Public Health unit, IPC, unpublished). If this incidence is applied to the rural population of Cambodia, a reasonable estimate of the national burden of dog bites would be at least 600,000 dog bites per year. Data on behavioral and other risk factors in dogs and human-canine contact associated with non-play dog bites in the Mekong Region are urgently needed[575].

6.2 PEP data in Cambodia

In Cambodia, three main centers currently propose PEP to the public, at a cost. The main center is located at Institut Pasteur du Cambodge, providing intradermal PEP to over 21,000 persons each year at an IPC-subsidized cost[71,576]. The center is primarily attended by patients from the ten main South-Eastern provinces, home to 2/3 of the Cambodian population (Figure

13). An additional 11,000 protocols may be administered each year nationwide, mainly in private healthcare settings, for a countrywide total of 32,000 PEP annually (approximately 5% of all dog bite victims)[71,576].

Figure 13: Cambodia, population density (dark) in Cambodia and cases referred to IPC's prevention center for PEP, 2009-2013 by district of residence (1 red dot = 10 patients).



6.3 Human rabies cases in Cambodia

There are no reliable surveillance data on human cases of rabies in Cambodia.

A model-based estimate of rabies deaths in Cambodia was performed by IPC epidemiologists on 2007 data, extrapolating figures from Phnom Penh to the rest of the country[70]. This study concluded to 810 human rabies deaths in 2007 (95%CI: 394-1,607), for an estimated incidence of 5.8/100,000 (95% CI: 2.8-11.5), the highest published figure worldwide and 3.5 times the estimated incidence in India[70,577]. In 2007, a total of 54 cases were notified to the national public health surveillance system in Cambodia, a 15-fold undernotification rate if modeling estimates are correct.

If these models are correct, this would translate to Cambodia suffering an estimated 1.3% of estimated rabies deaths worldwide, for a population of only 15 million, which is less than 0.2% of the population of affected countries[18,70,578]. This includes risk to foreign residents, as shown by a case of confirmed rabies in a French expatriate in June 2015[579].

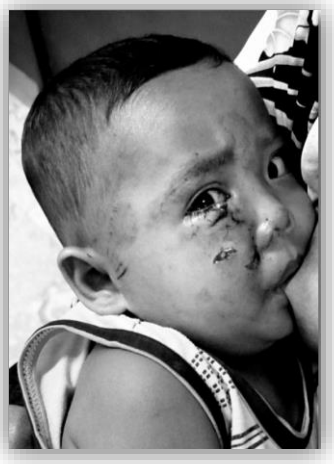


Figure 14: A Cambodian child bitten to the face by a dog with confirmed rabies, IPC, 2011. He survived after receiving PEP using intradermal vaccine and rabies immunoglobulin. (Photo A. Tarantola).

Since 2007, the Cambodian population has grown by an estimated 1.4 million. The canine population has grown as well (by at least 373,000 dogs if the 3:1 ratio in rural areas applies), with no routine canine vaccination program implemented as of January 2018. The annual number of human rabies deaths has therefore likely risen in Cambodia since 2007.

If we examine this estimated incidence of human rabies deaths per 100,000 pop. rather than the absolute number of cases, Cambodia suffers the 8th highest estimated rabies incidence in the World, the 3rd highest in Asia and possibly the highest in SE Asia (as the number of published estimated cases in Myanmar[18] seems disproportionate with the number of tested cases). Cambodia has pledged to comply with the ASEAN plan to eliminate canine and canine-mediated rabies in the Region by 2030[100,356–359,576].

6.4 Economic burden in Cambodia

Switching from nerve-based vaccines to cell-based vaccines (Section 11) led to an explosion in vaccine costs in Cambodia as elsewhere[11]. In 2012, the average wholesale price for one dose of the available cell-based vaccines licensed in Cambodia was US$7.90 (median US$8.10; range US$6.40 - US$8.90) while the wholesale price for one dose of ERIG ranged between US$20.00 and US$35.00[71].

In another, unpublished study conducted at IPC in 2013, the mean cost for a one-way bus/taxi trip to Phnom Penh for patients receiving PEP at IPC's rabies prevention clinic ranged from US$ 0 - US$ 22.5, with a mean value of US$ 3.11 ± 0.01. It was US$ 3.6 ± 0.06 among protocol noncompleters compared to US$ 3.06 ± 0.01 among completers (p<000).

The total out-of-pocket expenditure for four return trips (one person) added to the cost of vaccine (10 US$ for a full intradermal, 4-session IPC-subsidized protocol) and ERIG if needed

therefore amounted in 2012 to a mean value of US$ 64. Children had to be accompanied by a parent, doubling transportation costs. These out-of-pocket expense estimates exclude the cost of a tetanus booster and that of rabies confirmation in the dog, both borne by IPC. In 2012 the mean monthly income *per capita* was US$ 44.5 in rural areas of Cambodia[580].

The loss of income during travel and stay in the capital is an additional concern. The rise in PEP noncompletion during the rice harvest indicates that Cambodian rural dwellers hesitate to refer for PEP and risk jeopardizing their and their family's annual revenue from harvesting paddy[111].

7. Author's knowledge translation on rabies since November 2014

The author first registered in the PhD program in 11/2014. During the time that this PhD work was being conducted and written up, the author contributed to this and several other aspects of rabies prevention through communication and knowledge translation[581–583], a crucial component of research, especially in developing countries.

Other work conducted on the end-of-life management of patients with clinically-declared rabies and not included in this PhD was published[117,118], shared with WHO experts and endorsed in a workshop and restitution session chaired by this author during a 26-28 April 2017 meeting in Bangkok, Thailand. Like the IPC regimen, these new recommendations on palliative care are included in a chapter (Chapter 6) edited by this author in the Third report of the WHO Expert Consultation on Rabies (WHO TRS N°1012) released in April 2018[114]. The author also participated to workshops and subsequent writing of chapters on perspectives for research on rabies, on the risks and indications of rabies post-exposure prophylaxis and on modelling rabies PEP data.

The author's participation to nine SAGE working group on rabies meetings between September, 2016 and October 2017 contributed to extensive literature reviews and assessments. These were also shared on various themes during the WHO rabies experts 26-28 April 2017 meeting in Bangkok, Thailand. The author will co-author several publications born of these literature reviews, including on cost-effectiveness modeling (Hampson K. *et al.,* submitted; Trotter C. *et al.,* submitted), clinical evaluation of rabies PEP regimens and vaccines (Tarantola A. *et al.,* submitted), a review of PEP (Kessels J. *et al.* submitted) or the estimation of rabies deaths despite or in absence of PEP (Tarantola A. *et al.,* manuscript in preparation). Other articles

have been authored or co-authored since 11/2014 on the history of concepts associated with rabies[1‡], on the risks of mother-to-child rabies virus transmission[32], on the use of various rabies diagnostic techniques according to various health objectives[59], on the incidence of dog bites in Cambodia[67], on a case of rabies in a French expatriate in Cambodia[76], and on the phylogeny of rabies viruses isolated in Cambodia[474].

The author was kindly asked to contribute to Pasteur regional courses on rabies in Phnom Penh, Cambodia (27/10-7/11/2013), in Yaoundé, Cameroon (25/10-05/11/2016) and in Tehran, Iran (8-19 October 2017), with presentations on data management and analysis and/or on rabies palliative care. In all, over 100 rabies stakeholders from Asia and Africa attended.

During the Pasteur regional course in Phnom Penh, a document initially compiled by the author, shared with trainees and improved upon provides a detailed list of updated knowledge gaps on rabies and rabies research. This will help guide future rabies research in the Institut Pasteur International Network (IPIN). A rabies clinic blueprint (tools and features) was also shared and improved upon which will serve as a checklist to equip and operate peripheral rabies clinics, in Battambang, elsewhere in Cambodia and beyond.

As the then-head of the epidemiology and public health unit at IPC, the author repeatedly provided expertise to the Communicable Disease Control department of the Ministry of Health and the joint Ministry of Health/ministry of Agriculture Technical Working Group on Zoonoses, Cambodia. Data on rabies, on the optimal positioning of peripheral rabies clinics or on the direct and indirect benefits of shortening PEP regimens were shared in this way.

The literature review necessary to fully embrace the breadth of research and knowledge on rabies for this PhD has been organized into 12 chapters on historical, virological, biological, diagnostic, clinical and vaccine-related aspects of rabies. These may be published as an Open-Access manual, translating research data into an operational companion handbook for public health decisionmakers seeking to implement the 2030 strategy worldwide.

The author also contributed to lay communications for the general public during the time of the PhD. Several articles were written in the French- or English- language press in Cambodia. A case of rabies in a French expatriate in Cambodia was communicated through Promed[579] and is also accessible in French on the web.[§] The author helped review and contributed to a book on rabies and its prevention written by IPC colleagues in Khmer, targeting children in Cambodia. The author set up publicity events for World Rabies Day to disseminate prevention messages

‡ And unearthing several documents, including a detailed report of the Joseph Meister incident, now available on the IPC website at http://www.webcitation.org/6lAInzjOc

§ https://www.vidal.fr/actualites/15828/la_rage_au_cambodge_point_de_situation/

throughout Cambodia, speaking on radio and creating a Facebook page on rabies prevention with messages in English and in Khmer on World Rabies Day, September 28, 2013. As of September 28, 2015, a message disseminated through the Ministry of Health Facebook account reached 398,768 people and was shared, commented or "liked" 34,542 times, a use of mass media to hopefully inform and change health behaviour[583]. Further "boosting" thanks to a limited grant disseminated the message to a total of 3,816,744 people throughout Cambodia and other Asian countries over the following 28-day period, with 62,375 "likes".

Figure 15: Public advisory by Institut Pasteur du Cambodge of the opening of a first peripheral rabies prevention center in Battambang, Cambodia

Institut Pasteur du Cambodge

Institut Pasteur Du Cambodge

NEWSLETTER

JUNE 2018

NEWS

Rabies Prevention Center opening in BATTAMBANG in July 2018

We are very pleased to open soon in July our second Rabies Prevention Center, with Provincial Health Department Battambang (PHD), aiming to vaccinate 10 000 bitten people per year. The center will use the same standard and price as the Pasteur Institute in Phnom Penh (15USD full vaccination protocol in one week).

In Cambodia, we still have around 800 human deaths a year from rabies, and 50% under the age of 15.
The disease is 100% lethal if no vaccination, and that rabies is 100% preventable by vaccination after a bite injury. Therefore, Institut Pasteur du Cambodge and the Ministry of Health, with the support from different partners, initiated a five-year action plan to fight rabies in Cambodia.
In collaboration with the Ministry of Health, the Ministry of Agriculture, Forestry and Fisheries and the Ministry of Education, Youth and Sport, we aim to reduce rabies mortality in Cambodia by 50% in 5 years and save 2,000 lives in 5 years.
We want to increase access to vaccination after bite injuries by creating two rabies vaccination centers located in two provinces outside the capital city; one center in Battambang province and another center in the east of Phnom Penh (in 2019).

KNOW MORE
FACEBOOK

http://www.webcitation.org/712SJqoi8

Résumé

La rage entraîne plus de 60,000 décès par an dans le Monde, dont 800 au Cambodge, pays fortement endémique pour la rage canine.

La mort survient dans près de 100% des cas de rage, maladie évitable dans presque 100% des cas par l'accès à une prophylaxie post-exposition (PPE) antirabique adéquate et en temps utile. L'amélioration de l'accès à une PPE dans les zones rurales des pays endémiques permettra d'épargner des vies humaines à court terme.

Cette thèse en épidémiologie a tiré parti des données collectées auprès des patients consultant au centre antirabique et les chiens testés à l'Institut Pasteur du Cambodge (IPC), Phnom Penh. Suite à un bilan épidémiologique de la situation et des obstacles auxquels sont confrontés les patients cherchant à accéder à la PPE adéquate et en temps utile, elle vise à contribuer à améliorer 1/ l'accès géographique et 2/ l'accès financier à une PPE pour les populations rurales du Cambodge.

Nous avons développé une stratégie originale d'identification des poches de populations à haut risque d'incomplétude vaccinale après une exposition potentielle à la rage. Ceci devrait permettre d'améliorer l'accès géographique à la PPE et se concrétiser par l'ouverture en Juillet 2018 d'un centre périphérique de prévention de la rage dans l'Ouest du Cambodge. Cette stratégie d'identification de difficultés d'accès aux soins est applicable à d'autres thématiques de santé, sous certaines conditions.

Notre rappel des patients et l'analyse des décès par rage parmi les patients n'ayant pas complété de leur propre chef le protocole PPE de 4 sessions intradermales sur 1 mois ne permettent pas de mettre en évidence une différence de mortalité par rage parmi les patients n'ayant reçu que 3 sessions sur 1 semaine, par rapport à au moins 4 sessions/1mois. Le raccourcissement du protocole à 1 semaine permet de réduire les coûts directs et indirects et l'absence de revenus pendant la durée du traitement en capitale. La mise en place de ce protocole doit s'accompagner d'un suivi d'au moins 6 mois des patients après leur prise en charge initiale.

L'ensemble de ces travaux a des implications qui dépassent le cadre du Cambodge: Dans ses recommandations d'Avril 2018, l'OMS recommande désormais ce nouveau protocole IPC– le premier protocole PPE antirabique abrégé à 1 semaine.

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