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# Epidemiology of leptospirosis in New Caledonia and Futuna: symptomatic infections in children, symptomatic reinfections and incidence of Jarisch-Herxheimer reactions

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Gilles Guerrier. Epidemiology of leptospirosis in New Caledonia and Futuna: symptomatic infections in children, symptomatic reinfections and incidence of Jarisch-Herxheimer reactions. Infectious diseases. Université Pierre et Marie Curie - Paris VI, 2017. English. NNT : 2017PA066012 . tel-01676711

**HAL Id: tel-01676711**

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**Dossier de**

**Validation des Acquis de l'Expérience**

**En vue de l'obtention d'un doctorat**

**Spécialité : Épidémiologie**

**École Doctorale Pierre Louis de Santé**

**Publique : Épidémiologie et Sciences de**

**l'Information Biomédicale (ED 393)**

**de l'UPMC**

Gilles Guerrier - 4 janvier 2017

## **Abbreviations**

AP-HP : Assistance Publique Hôpitaux de Paris

CHT: Centre hospitalier territorial

CT: Cranial traumatism

CRISPR: clustered regulatory interspaced palindromic repeats

DDH: DNA hybridization

GDP: Gross domestic product

HuScFv: Humanized-murine single-chain monoclonal antibody

Ig: Immunoglobulin

IL: Interleukine

JHR: Jarisch-Herxheimer reaction

Lip: Lipoprotein

LPS: Lipopolysaccharide

MAT: Microagglutination test

MSF: Médecins Sans Frontières

MLST: Multilocus sequencing typing

OMP: Outer membrane protein

PCR: Polymerase chain reaction

SNP: Single nucleotide polymorphisms

TNF: Tumor Necrosis Factor

VAE: Validation des acquis de l'expérience

WHO: World Health Organization

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## **Epidemiology of leptospirosis in New Caledonia and Futuna: symptomatic infections in children, symptomatic reinfections and incidence of Jarisch-Herxheimer reactions**

This thesis is based on the following papers :

1. Guerrier G, Hie P, Gourinat AC, Huguon E, Polfrit Y, Goarant C, D'Ortenzio E, Missotte I. Association between age and severity to leptospirosis in children. *PLoS Negl Trop Dis*. 2013 Sep 26;7(9):e2436.
2. Guerrier G, D'Ortenzio E. The Jarisch-Herxheimer reaction in leptospirosis: a systematic review. *PLoS One*. 2013;8(3):e59266.
3. Guerrier G, Lefèvre P, Chouvin C, Eric D'Ortenzio E. Jarisch-Herxheimer reaction among patients with leptospirosis: incidence and risk factors. *Am J Trop Med Hyg* 2016 (submitted 5<sup>th</sup> July 2016).
4. Guerrier G, Chouvin C, Eric D'Ortenzio E. Reinfection in leptospirosis: a 3-year retrospective study in two Pacific islands. *Trans R Soc Trop Med* 2016 (submitted 24<sup>th</sup> Oct 2016).

## **II.1. Introduction**

Leptospirosis is a zoonosis of ubiquitous distribution, caused by infection with pathogenic *Leptospira* species. The spectrum of human disease caused by leptospire is extremely wide, ranging from subclinical infection to a severe syndrome of multiorgan infection with high mortality. This syndrome, icteric leptospirosis with renal failure, was first reported over 100 years ago by Adolf Weil in Heidelberg (Weil, 1886). However, an apparently identical syndrome occurring in sewer workers was described several years earlier (Landouzy, 1883). Leptospirosis was certainly recognized as an occupational hazard of rice harvesting in ancient China (Faine, 1994) and the Japanese name akiyami, or autumn fever, persists in modern medicine. With hindsight, clear descriptions of leptospiral jaundice can be recognized as having appeared earlier in the 19th century, some years before the description by Weil. The etiology of leptospirosis was demonstrated independently in 1915 in Japan and Germany (Everard, 1996). Ten years before, Stimson demonstrated by silver staining the presence of clumps of spirochetes in the kidney tubules of a patient who reportedly died of yellow fever (Stimpson, 1907). The spirochetes had hooked ends, and Stimson named them *Spirochaeta interrogans* because of their resemblance to a question mark.

## **II.2. Epidemiology**

### **II.2.1. Global morbidity and mortality of leptospirosis**

It is estimated that leptospirosis causes 1.03 (95% CI 0.43–1.75) million cases worldwide each year. These estimates place the disease among the leading zoonotic causes of morbidity and mortality. Table 1 illustrates the burden per 100,000 of leptospirosis compared to the estimates of other neglected and tropical diseases estimated by GBD 2010. Thus the burden of leptospirosis appears to be of a similar magnitude to that of schistosomiasis, leishmaniasis and lymphatic filariasis and about 73% of that of cholera. Furthermore, 2.90 million DALYs represent the equivalent of all the inhabitants of a city the size of Rome or Nairobi losing one year of healthy life. Furthermore, the number of estimated deaths (58,900; 95% CI 23,800–95,900) attributable to leptospirosis approaches or exceeds those for causes of haemorrhagic fever which were investigated in the Global Burden of Disease Study 2010 (Lozano, 2010) and other studies (Bhatt, 2013).

Diseases	DALYs per 100,000 [16].
Malaria	1200 (921–1594)
Cholera	65 (49–84)
Leishmaniasis	48 (32–71)
Schistosomiasis	48 (25–91)
Leptospirosis	42 (18.1–66)
Lymphatic filariasis	40 (26–58)
Rabies	21 (12–39)

**Table 1. The burden of leptospirosis per 100,000 compared to global burden diseases estimates for the burden of various tropical and neglected diseases (adapted from Torgerson, 2015).**

The large majority of the estimated disease burden occurred in tropical regions and the world's poorest countries (Table 2). Morbidity estimates reflect mainly the incidence of severe leptospirosis, rather than rates for clinical or symptomatic illness, since selected studies used case definitions that relied on detection of severe manifestations (Agampodi, 2011). Severe leptospirosis is generally believed to account for a small fraction (5–15%) of all clinical infections (Ko, 2009). Considering the annual number of deaths worldwide, the impact of leptospirosis equals that of canine rabies (59,000 annual deaths) (Hampson, 2015). The burden of leptospirosis, with respect to morbidity, is higher than some other important neglected tropical diseases, including visceral leishmaniasis and severe dengue, and is similar to others, including echinococcosis and cysticercosis (Hotez, 2014).

Although, the relatively poor data quality and quantity that inform the estimates in Costa *et al.* (2015) and Torgerson *et al.* (2015), as manifested by the wide confidence intervals, there is a

growing recognition that leptospirosis is an important cause of an acute febrile illness:

leptospirosis has been shown to be the cause of 5–69% of acute undifferentiated or non-malarial fever cases in different parts of the world (Mayxay, 2013; Crump, 2013; Reller, 2014).

Leptospirosis, as in the case of dengue (Bhatt, 2013), may therefore account for a much greater burden than indicated by morbidity estimates of severe disease. Moreover, the health outcomes of leptospirosis have been traditionally associated with its acute disease. The disease causes sub-acute and chronic complications, such as uveitis (Rathinam, 2002), and persistent complaints (Goris, 2013). However, the frequency and magnitude of long-term sequelae have not been rigorously quantified.

GBD region	Morbidity <sup>a</sup>	Cases	Mortality <sup>a</sup>	Deaths
	Estimate (95% confidence interval of the prediction)			
All GBD regions	14.77 (4.38–25.03)	1,030,000 (434,000–1 750,000)	0.84 (0.34–1.37)	58,900 (23,800–95,900)
High Income Asia Pacific	6.95 (2.51–11.87)	14,800 (5,300–25,100)	0.31 (0.13–0.52)	700 (300–1,100)
Central Asia	5.53 (2.07–9.51)	4,400 (1,600–7,300)	0.29 (0.12–0.49)	200 (100–400)
East Asia	10.28 (3.58–18.28)	142,000 (49,400–252,000)	0.50 (0.19–0.88)	6,900 (2,600–12,200)
South Asia	17.97 (6.20–32.25)	289,000 (99,800–519,000)	1.02 (0.41–1.71)	16,500 (6,500–27,600)
South–East Asia	55.54 (20.32–99.53)	266,000 (97,500–477,000)	2.96 (1.65–4.93)	14,200 (5,600–24,000)
Australasia	9.13 (2.79–16.36)	2,400 (700–4,200)	0.40 (0.16–0.69)	100 (0–200)
Caribbean	50.68 (14.93–87.58)	22,300 (6,700–34,700)	2.90 (1.14–4.72)	1,300 (500–1,900)
Central Europe	4.02 (1.21–6.85)	4,800 (1,500–8,200)	0.21 (0.08–0.33)	200 (100–400)
Eastern Europe	1.43 (0.51–2.69)	2,900 (1,100–5,500)	0.09 (0.04–0.16)	200 (100–300)
Western Europe	3.90 (1.45–6.49)	16,300 (6,100–27,100)	0.18 (0.07–0.29)	800 (300–1,200)
Andean Latin America	21.90 (7.92–39.82)	11,700 (4,200–21,200)	0.96 (0.37–1.68)	500 (200–900)
Central Latin America	15.77 (5.83–27.37)	36,000 (12,300–62,400)	0.68 (0.27–1.15)	1,600 (600–2,600)
Southern Latin America	3.87 (1.43–6.74)	2,400 (900–4,100)	0.18 (0.07–0.31)	100 (0–200)
Tropical Latin America	13.53 (4.47–26.56)	27,300 (9,000–53,200)	0.66 (0.23–1.28)	1,300 (500–2,600)
North Africa / Middle East	7.30 (2.58–11.79)	33,300 (11,800–53,800)	0.34 (0.14–0.56)	1,600 (600–2,500)
High Income North America	3.64 (1.02–6.50)	12,800 (3,600–22,900)	0.18 (0.07–0.31)	600 (200–1,100)
Oceania	150.68 (40.32–272.29)	16,700 (4,500–30,200)	9.61 (3.56–17.11)	1,100 (400–1,900)
Central Sub–Saharan Africa	13.49 (4.48–23.56)	13,100 (4,400–22,900)	1.33 (0.52–2.23)	1,300 (500–2,200)
East Sub–Saharan Africa	25.65 (9.29–43.31)	91,100 (33,000–154,000)	1.87 (0.79–3.12)	6,700 (2,800–11,100)
Southern Sub–Saharan Africa	3.44 (1.34–5.78)	2,400 (900–4,100)	0.33 (0.13–0.53)	200 (100–400)
West Sub–Saharan Africa	9.67 (3.62–16.16)	32,000 (12,000–53,500)	0.85 (0.35–1.36)	2,800 (1,200–4,500)

<sup>a</sup>Annual leptospirosis morbidity and mortality rates are shown as cases or deaths, respectively, per 100,000 population.

**Table 2. Estimated annual leptospirosis morbidity and mortality according to GBD region (adapted from Costa, 2015).**

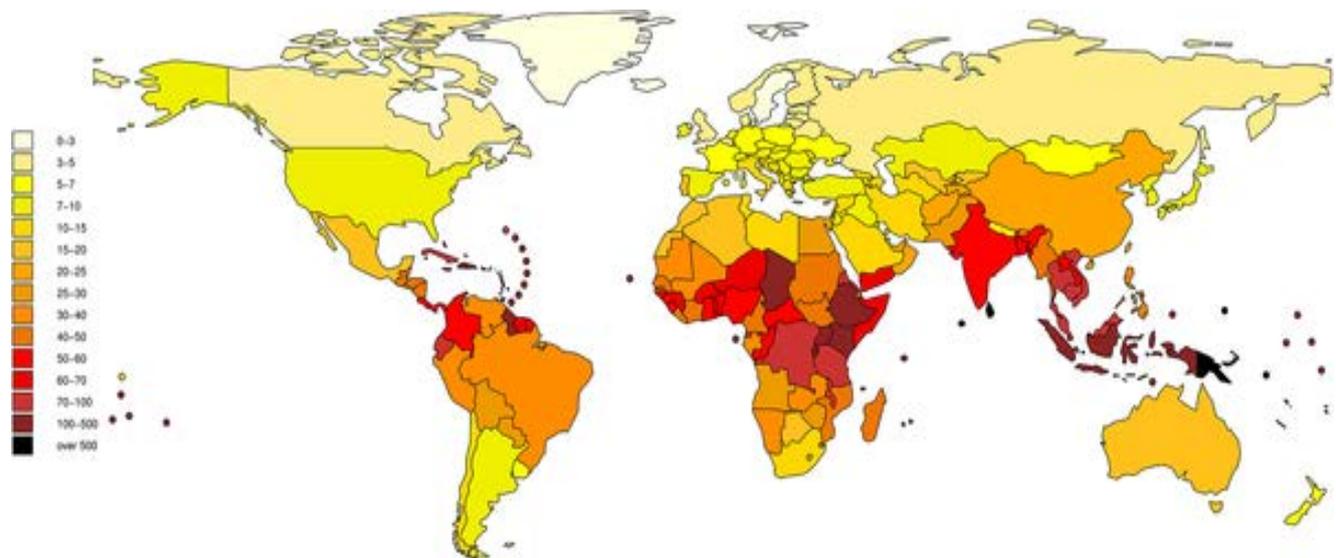
Regions within the developing world where the burden of leptospirosis may be significantly under-recognized include Southeast Asia and Oceania (Table 3). Although transmission is endemic and large outbreaks have been reported in these regions (Punjabi, 2012), active surveillance for leptospirosis has not been routinely performed.

Country	Morbidity <sup>a</sup> (95% CI)	Mortality <sup>a</sup> (95% CI)
<b>Western Pacific region, stratum B</b>		
Cambodia	33·65 (10·68 – 63·81)	1·83 (0·65 – 3·35)
China	10·54 (3·52 – 18·39)	0·50 (0·19 – 0·89)
Fiji	54·38 (18·35 – 101·12)	3·08 (1·25 – 5·50)
Kiribati	106·25 (36·04 – 194·67)	6·23 (2·28 – 10·96)
Lao People's Democratic Republic	19·11 (6·61 – 34·07)	1·09 (0·44 – 1·91)
Malaysia	36·98 (11·36 – 73·20)	1·68 (0·64 – 3·42)
Marshall Islands	25·71 (9·23 – 47·42)	2·23 (0·90 – 4·15)
Micronesia, Federated States of	245·09 (77·30 – 490·19)	12·42 (4·59 – 22·37)
Mongolia	2·65 (0·87 – 5·07)	0·14 (0·05 – 0·25)
Nauru	19·90 (5·48 – 44·02)	1·93 (0·65 – 3·98)
Palau	64·06 (20·93 – 120·03)	3·80 (1·38 – 7·30)
Papua New Guinea	195·22 (61·38 – 370·88)	12·48 (4·68 – 24·09)
Philippines	14·98 (4·94 – 26·91)	0·74 (0·26 – 1·31)
Republic of Korea	5·02 (1·69 – 8·77)	0·22 (0·08 – 0·39)
Samoa	136·69 (47·42 – 274·47)	7·65 (3·08 – 14·22)
Solomon Islands	262·93 (84·11 – 518·76)	12·11 (4·37 – 22·80)
Tonga	116·07 (38·15 – 225·64)	5·92 (2·17 – 11·28)
Tuvalu	74·23 (24·60 – 131·78)	5·03 (1·86 – 8·72)
Vanuatu	121·15 (41·99 – 233·46)	6·41 (2·45 – 11·79)
Viet Nam	49·69 (16·47 – 98·92)	2·09 (0·79 – 4·15)
Non-independent colonies, states, and territories		
<i>French Polynesia</i>	97·49 (32·20 – 179·82)	4·55 (1·67 – 8·01)
<i>Hong Kong</i>	10·37 (3·28 – 20·19)	0·42 (0·15 – 0·83)
<i>Macao</i>	9·72 (3·02 – 19·03)	0·37 (0·13 – 0·71)
<i>New Caledonia</i>	68·53 (22·89 – 124·93)	2·95 (1·13 – 5·36)
<i>Taiwan</i>	13·14 (4·60 – 24·44)	0·54 (0·20 – 0·97)
<i>Wallis and Funtura</i>	745·65 (209·01 – 1754·34)	27·20 (8·41 – 61·99)

**Table 3. Estimated leptospirosis morbidity and mortality by country, grouped according to WHO sub-region (adapted from Costa, 2015).**

The reported incidence of leptospirosis reflects the availability of laboratory diagnosis and the clinical index of suspicion as much as the incidence of the disease. A knowledge of the prevalent serovars and their maintenance hosts is essential to understanding the epidemiology of the disease in any region. Hospital-based surveillance studies, which capture more severe forms leading to hospitalization, do not measure mild febrile forms that form the base of the iceberg of leptospirosis cases. The global burden of leptospirosis is estimated at 2.90 million DALYs per annum (1.25–4.54 million). This consisted of 2.80 million YLLs (1.16 million–4.46 million) and 103,200 (38,800–188,100) YLDs. This represents an incidence of 41.8 DALYs per 100,000 population per

year (18.1–65.5). The frequency distribution of the global DALY estimate is illustrated in Figure 1. Males are predominantly affected with an estimated 2.33 million DALYs (0.95–3.66 million) or approximately 80% of the total burden. Young adults aged 20–49 had an estimated burden of 1.5 million DALYs (0.65–2.32 million) or approximately 52% of the total. Of these young men age 20–49 have a burden of 1.30 million (0.56–2.03 million) or 45% of the total burden.



**Figure 1. Global burden of leptospirosis: estimated in terms of disability adjusted life years (adapted from Torgerson, 2015).**

## **II.2.2. Environmental risk factors of infection**

Geography, climate, and poverty are the main determinants in the worldwide distribution of leptospirosis. Countries situated in the tropics had the highest estimated disease incidence and accounted for 73% of the world’s estimated cases. This pattern is attributable to environmental and social conditions which promote the abundance of reservoir animals, survival of the bacterium in soil and surface water, and risk of human exposures with these sources of infection (Reis, 2008). Tropical climate favours transmission of leptospirosis, which is often seasonal and increases during periods of heavy rainfall (Lau, 2010). The disease is seasonal, with peak incidence occurring in summer or fall in temperate regions, where temperature is the limiting factor in survival of leptospire, and during rainy seasons in warm-climate regions, where rapid desiccation would otherwise prevent survival. The disease is well-recognized as a health problem of impoverished rural-subsistence farmers (Sethi, 2010), pastoralists (Biggs, 2013), and urban slum dwellers (Maciel, 2008). Life expectancy, which serves in part as a proxy for poverty, is an independent predictor of country-specific disease incidence (Table 4). Although urban slum environments are an emerging and increasingly important setting for leptospirosis transmission

(Maciel, 2008), country percent urbanization is inversely associated with leptospirosis incidence, reflecting in part the high burden of leptospirosis in rural settings, but also the well-recognized association between lower aggregated country-level percent urbanization and poverty.

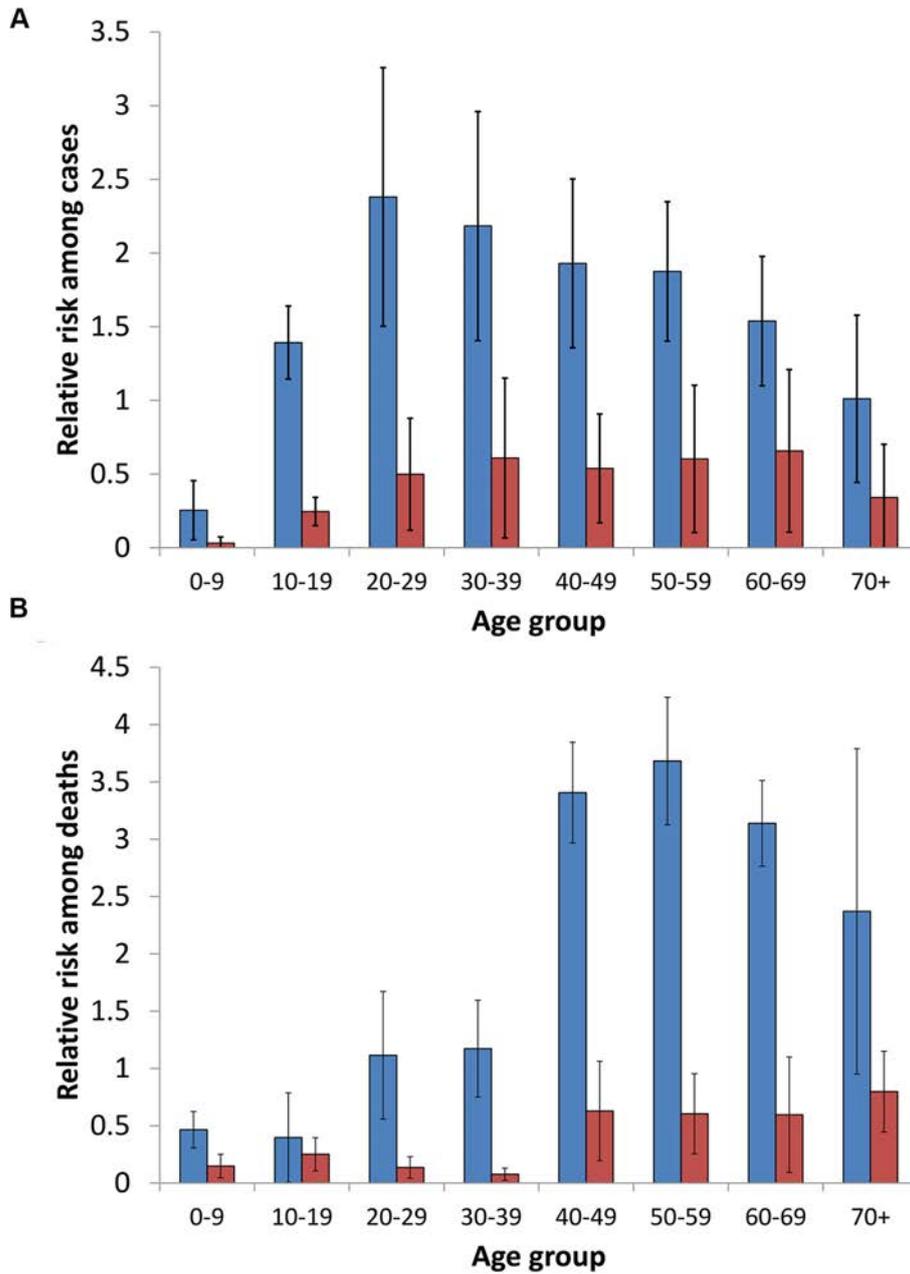
Variable <sup>a</sup>	R Squared
Distance from the equator	0.105
Percent urbanization of population	0.243
Life expectancy at birth	0.405
Tropical island	0.478
Complete model	0.600 <sup>b</sup>

**Table 4. Variables independently associated with leptospirosis morbidity in 34 countries (adapted from Costa, 2015).**

Although leptospirosis is often considered to be a rural disease, people living in cities may also be exposed, notably to rats. The risk of such exposure will depend on the living conditions and the level of hygiene both in the house and its immediate environment, and on the general hygienic and sanitary conditions in different areas of a city. By 2037, the majority of the world's population will be inhabitants of urban centres in developing countries. A large proportion of this population will reside in slum settlements, where poor sanitation has created the conditions for annual rainfall-associated epidemics (Riley, 2007). Extreme weather events and flood-related disasters (Lau, 2010) are predicted to escalate with global climate change (Patz, 2005). As deforestation and agricultural expansion intensify in tropical regions (De Fries, 2010), rural-based farming populations may be increasingly exposed to leptospirosis.

### II.2.3. Individual risk factors of transmission

According to Costa et al., the risk of acquiring leptospirosis is higher in adults than children and higher in males than females, and highest among adult males of 20 to 29 years of age (Figure 2A). The age and gender-specific risk for death has a different pattern: the risk for death increases with increasing age (Figure 2B) (Costa, 2015). Unfortunately, raw data for calculating age and gender specific risk for disease and death were extracted from grey literature by Costa et al (see Annexes and Costa, 2015) limiting our ability to formulate a critical appraisal of those figures. However, male gender preference is a well-recognized phenomenon in leptospirosis and due to the gender-specific occupational and peri-domiciliary risk activities (Bovet, 1999). Morbidity and mortality in the Western Pacific region (WHO sub-region) stratified by age and gender is shown in Table 5.



**Figure 2. Mean relative risk for membership in age and gender groups among 6011 leptospirosis cases (A) and 164 deaths (B) from compiled data in 10 countries. Mean and standard deviation of the relative risks are presented for males (blues bars) and females (red bars) (adapted from Costa, 2015).**

Demographic	Morbidity (95% CI)	Cases (95% CI)	Mortality (95% CI)
<b>Western Pacific region, stratum B</b>			
Females			
0 – 9	0.51 (0.16 – 1.04)	544 (172 – 1104)	0.13 (0.05 – 0.26)
10 – 19	3.71 (1.17 – 7.86)	4510 (1417 – 9565)	0.20 (0.07 – 0.41)
20 – 29	6.95 (2.05 – 13.74)	9328 (2746 – 18440)	0.10 (0.03 – 0.21)
30 – 39	8.28 (2.42 – 18.58)	10463 (3054 – 23494)	0.05 (0.02 – 0.11)
40 – 49	6.91 (2.27 – 14.47)	8962 (2941 – 18770)	0.41 (0.14 – 0.87)
50 – 59	7.60 (2.43 – 16.00)	7080 (2268 – 14910)	0.38 (0.12 – 0.78)
60 – 69	8.03 (2.61 – 16.74)	4432 (1442 – 9242)	0.35 (0.12 – 0.76)
70 and older	4.18 (1.23 – 9.13)	1952 (575 – 4265)	0.46 (0.15 – 0.93)
Males			
0 – 9	3.99 (1.28 – 7.91)	4987 (1601 – 9889)	0.38 (0.14 – 0.74)
10 – 19	21.32 (6.57 – 43.98)	29703 (9161 – 61280)	0.30 (0.10 – 0.61)
20 – 29	33.54 (10.40 – 69.81)	48248 (14955 – 100430)	0.77 (0.28 – 1.48)
30 – 39	30.22 (9.45 – 61.67)	39761 (12433 – 81127)	0.79 (0.26 – 1.63)
40 – 49	24.78 (7.16 – 53.44)	33299 (9620 – 71814)	2.13 (0.73 – 4.33)
50 – 59	23.89 (6.99 – 52.34)	22735 (6647 – 49794)	2.21 (0.70 – 4.63)
60 – 69	18.32 (5.20 – 40.10)	10192 (2894 – 22313)	1.82 (0.59 – 3.77)
70 and older	11.88 (3.73 – 25.70)	4684 (1471 – 10135)	1.38 (0.45 – 3.10)

**Table 5. Estimated age group and gender-specific leptospirosis morbidity and mortality in the Western Pacific WHO sub-region (adapted from Costa, 2015).**

Exposure depends on chance contacts between humans and infected animals or a contaminated environment. The names for some forms of leptospirosis (e.g. rice field fever, cane cutter's disease, swineherd's disease, dairy farm fever, mud fever) reflect transmission conditions. The degree and nature of exposure often depend on occupational and/or recreational and social activities (Bovet, 1999). Cattle farmers may be exposed when handling cattle, notably during milking, or when touching dead, aborted fetuses and other procreational products, e.g. amniotic fluid or placenta, or if they come into contact with infectious droplets when cows urinate. Pig farmers may be exposed when tending pigs.

Vegetable farmers and gardeners may be directly or indirectly exposed to infected rodents or their urine. Farmers may be exposed to water contaminated by the urine of rodents or other animals when irrigating fields. Rice farmers, particularly when barefooted, may be exposed to water contaminated by rodents or buffaloes, e.g. when ploughing. Veterinarians and pet keepers may be exposed to infected animals that are ill or have died from leptospirosis or which may be symptomless carriers/shedders (this also applies to cattle and pig farmers). Abattoir workers and butchers may be exposed when slaughtering infected animals and handling infected carcasses and organs, e.g. kidneys. Those involved in food preparation may be exposed to rat-contaminated surroundings when hygienic measures are unsatisfactory. Sewer workers may be exposed to

sewage contaminated by rat urine. Sugar cane workers may be exposed when cutting sugar cane that is contaminated by urine of rodents living in the cane fields. Miners may be exposed to water contaminated with rat urine in mines. People may be exposed when herding cattle. People living in close contact with domestic animals may be exposed if these animals are infected. Inland fishermen and fish/prawn farmers may be exposed, particularly if the environment and surface waters are contaminated. Soldiers, hunters and hikers may be exposed when wading through contaminated surface waters or swamps, walking on or through contaminated soil, mud or wet vegetation, or by contact with animals. Children may be exposed when playing in yards (in rain puddles or mud) contaminated by animals, such as dogs, pigs, and rats. Those participating in leisure and recreational activities (swimming, sailing, canoeing, rafting, caving, canyoneering, fishing, etc.) or involved in accidents (car accidents, boat accidents) may come into contact with contaminated surface waters, especially with prolonged (head under water) submersion. This also applies to travellers participating in jungle adventure trips or outdoor sports activities. Entire villages may be exposed when the drinking-water source is inadequately treated. Laboratory staff involved in the diagnosis of and research on leptospirosis and other zoonotic research, and especially field workers are at risk of exposure.

#### **II.2.4. Mechanisms of transmission**

The source of infection in humans is usually either direct or indirect contact with the urine of an infected animal. Animals may be maintenance hosts of some serovars but incidental hosts of others, infection with which may cause severe or fatal disease. **It is believed that the most important maintenance hosts are small mammals (Babudieri, 1958).** which may transfer infection to domestic farm animals, dogs, and humans. A particular *Leptospira* strain will generally have a preference for a certain animal host with which there is a largely commensal relationship: the host does not suffer or suffers only comparatively mildly from the infection. Different rodent species may be reservoirs of distinct serovars, but rats are generally maintenance hosts for serovars of the serogroups Icterohaemorrhagiae and Ballum, and mice are the maintenance hosts for serogroup Ballum. Domestic animals are also maintenance hosts; dairy cattle may harbor serovars hardjo, pomona, and grippotyphosa; pigs may harbor pomona, tarassovi, or bratislava; sheep may harbor hardjo and pomona; and dogs may harbor canicola (Table 6). Distinct variations in maintenance hosts and the serovars they carry occur throughout the world.

Reservoir host	Serovar(s)
Pigs	<i>pomona, tarassovi</i>
Cattle	<i>hardjo, pomona</i>
Horses	<i>bratislava</i>
Dogs	<i>canicola</i>
Sheep	<i>hardjo</i>
Raccoon	<i>grippotyphosa</i>
Rats	<i>icterohaemorrhagiae, copenhageni</i>
Mice	<i>ballum, arborea, bim</i>
Marsupials	<i>grippotyphosa</i>
Bats	<i>cynopteri, wolffi</i>

**Table 6. Typical reservoir hosts of common leptospiral serovars (adapted from Bharti, 2003).**

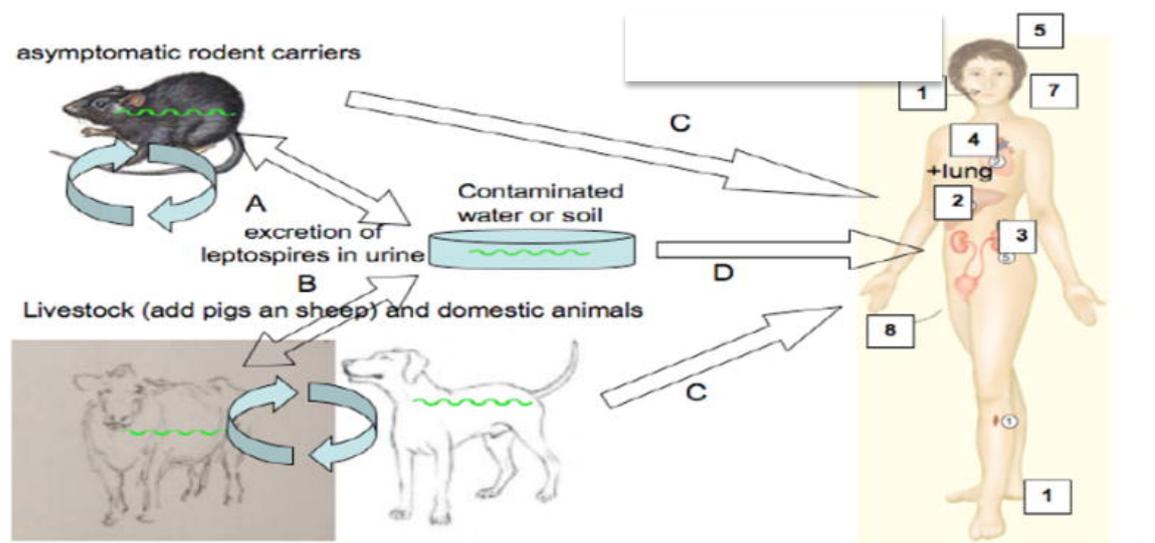
Animals, including humans, can be divided into maintenance hosts and accidental (incidental) hosts. The disease is maintained in nature by chronic infection of the renal tubules of maintenance hosts (Badudieri, 1958). A maintenance host is defined as a species in which infection is endemic and is usually transferred from animal to animal by direct contact **via their urine**. Infection is usually acquired at an early age, and the prevalence of chronic excretion in the urine increases with the age of the animal. Other animals (such as humans) may become infected by indirect contact with the maintenance host. Leptospire live and multiply in the kidneys of a carrier (generally mammalian) or maintenance animal host. They are excreted in the urine, and animals that excrete leptospire are called shedders. Infected animals transfer leptospire to their offspring either in utero or during the neonatal period. These offspring then transfer the infection to their own offspring, and so on. In this way, a chain of infection is maintained by the maintenance host. By maintaining the infection, such maintenance hosts form the reservoir of infection. The distinction between natural or maintenance and incidental/accidental hosts is not always clear cut, particularly in domestic animals that are kept in crowded conditions that favour transmission. Exceptions and transitional states may also occur. Leptospire may adapt to a new host that may eventually become a natural maintenance host when the infection establishes itself in a population of animals of the same species, thus forming an infection reservoir. An animal may be temporarily carrying and shedding leptospire without being a natural maintenance host if the infection does not establish itself in a population of animals of the same species. The infection may run a chronic course in the maintenance host with or without serious sequelae.

The usual portal of entry is through abrasions or cuts in the skin or via the conjunctiva; infection may take place via intact skin after prolonged immersion in water, but this usually occurs when abrasions are likely to occur and is thus difficult to substantiate. Human infections may be

acquired through occupational, recreational, or avocational exposures. Occupation is a significant risk factor for humans (Waitkins, 1986). Direct contact with infected animals accounts for most infections in farmers, veterinarians, abattoir workers, meat inspectors, rodent control workers, and other occupations which require contact with animals. In tropical wet areas, there are many serovars infecting humans and animals and larger numbers of reservoir species, including rodents, farm animals, and dogs. Human exposure is not limited by occupation but results more often from the widespread environmental contamination, particularly during the rainy season. Control of rodent populations, drainage of wet areas, and occupational hygiene are all necessary for prevention of human leptospirosis. Pathogenic serovars have been isolated from water in tropical regions (Alexander, 1975). Survival of pathogenic leptospires in the environment is dependent on several factors, including pH, temperature, and the presence of inhibitory compounds. Many infections result from barefooted walking in damp conditions or gardening with bare hands (Douglin, 1997).

Mammalian species excrete the pathogen in their urine and serve as reservoirs for transmission. The pathogen is maintained in sylvatic and domestic environments by transmission among rodent species. In these reservoirs, infection produces chronic and persistent asymptomatic carriage in the renal tubules where *L. interrogans* forms aggregates (Figure 3). Leptospires infect livestock and domestic animals and causes a range of disease manifestations and carrier states. Maintenance of leptospirosis in these populations is due to continued exposure to rodent reservoirs or transmission within animal herds.

Leptospirosis is transmitted to humans by direct contact with reservoir animals or exposure to environmental surface water or soil contaminated with their urine. Leptospires penetrate abraded skin or mucous membranes, infect the bloodstream and disseminate throughout all the body tissue. Infection causes an acute febrile illness during the early “leptospiraemic” phase, which progresses during late “immune” phase to cause severe multi-system manifestations such as hepatic dysfunction and jaundice, acute renal failure, pulmonary haemorrhage syndrome, myocarditis and meningoencephalitis. Although the immune response eventually eliminates the pathogen, leptospires may persist for prolonged periods in immunoprivileged sites, such as the anterior chamber and vitreous of the eye and the renal tubules, where they can produce respectively, uveitis months after exposure and urinary shedding weeks after resolution of the illness. Humans are an accidental host and **it is assumed they do not efficiently shed sufficient numbers of leptospires to serve as reservoirs for transmission** (Figure 3).



**Figure 3. Cycle of infection (adapted from Ko, 2009).**

## II.3. Bacteriology

### II.3.1. Serological classification

Prior to 1989, the genus *Leptospira* was divided into two species, *L. interrogans*, comprising all pathogenic strains, and *L. biflexa*, containing the saprophytic strains isolated from the environment. *L. biflexa* was differentiated from *L. interrogans* by the growth of the former at 13°C and growth in the presence of 8-azaguanine (225 µg/ml) and by the failure of *L. biflexa* to form spherical cells in 1 mL NaCl.

Both *L. interrogans* and *L. biflexa* are divided into numerous serovars defined by agglutination after cross-absorption with homologous antigen. If more than 10% of the homologous titer remains in at least one of the two antisera on repeated testing, two strains are said to belong to different serovars. Over 60 serovars of *L. biflexa* have been recorded (Kmetz, 1993). Within the species *L. interrogans* over 200 serovars are recognized; additional serovars have been isolated but have yet to be validly published. Serovars that are antigenically related have traditionally been grouped into serogroups (Yasuda, 1987). While serogroups have no taxonomic standing, they have proved useful for epidemiological understanding. The serogroups of *L. interrogans* and some common serovars are shown in Table 7.

Serogroup	Serovar(s)
icterohaemorrhagiae, .....	Icterohaemorrhagiae, copenhageni, lai, zimbabwe
Hebdomadis .....	hebdomadis, jules, kremastos
Autumnalis .....	autumnalis, fortbragg, bim, weerasinghe
Pyrogenes.....	pyrogenes
Bataviae .....	bataviae
Grippotyphosa.....	grippotyphosa, canalzonae, ratnapura
Canicola .....	canicola
Australis.....	australis, bratislava, lora
Pomona .....	pomona
Javanica.....	javanica
Sejroe .....	sejroe, saxkoebing, hardjo
Panama .....	panama, mangus
Cynopteri .....	cynopteri
Djasiman.....	djasiman
Sarmin .....	sarmin
Mini .....	mini, georgia
Tarassovi.....	tarassovi
Ballum .....	ballum, aroborea
Celledoni.....	celledoni
Louisiana .....	louisiana, lanka
Ranarum .....	ranarum
Manhao .....	manhao
Shermani.....	shermani
Hurstbridge.....	hurstbridge

**Table 7. Serogroups and some serovars of *L. interrogans* sensu lato (adapted from Bharti, 2003).**

### II.3.2. Genotypic classification

The phenotypic classification of leptospire has been replaced by a genotypic one, in which a number of genomospecies include all serovars of both *L. interrogans* and *L. biflexa*. After an extensive study of several hundred strains, workers at the Centers for Disease Control defined 16 genomospecies of *Leptospira* that included those described previously (Ramadass, 1992) and adding five new genomospecies (Brenner, 1999), one of which was named *L. alexanderi*. DNA hybridization studies have also confirmed the taxonomic status of the monospecific genus *Leptonema* (Levett, 2014).

The genomospecies of *Leptospira* do not correspond to the previous *L. interrogans* and *L. biflexa*), and indeed, pathogenic and nonpathogenic serovars occur within the same species. Thus, neither serogroup nor serovar reliably predicts the species of *Leptospira*. Moreover, some studies have included multiple strains of some serovars and demonstrated genetic heterogeneity within serovars (Table 8). In addition, the phenotypic characteristics formerly used to differentiate *L. interrogans* sensu lato from *L. biflexa* sensu lato do not differentiate the genomospecies.

Serovar	Species
<i>bataviae</i>	<i>L. interrogans</i> , <i>L. santarosai</i>
<i>bulgarica</i>	<i>L. interrogans</i> , <i>L. kischneri</i>
<i>grippotyphosa</i>	<i>L. interrogans</i> , <i>L. kischneri</i>
<i>hardjo</i>	<i>L. borgpetersenii</i> , <i>L. interrogans</i> , <i>L. meyeri</i>
<i>icterohaemorrhagiae</i>	<i>L. interrogans</i> , <i>L. inadai</i>
<i>kremastos</i>	<i>L. interrogans</i> , <i>L. santarosai</i>
<i>mwogolo</i>	<i>L. interrogans</i> , <i>L. kischneri</i>
<i>paidjan</i>	<i>L. interrogans</i> , <i>L. kischneri</i>
<i>pomona</i>	<i>L. interrogans</i> , <i>L. noguchii</i>
<i>pyrogenes</i>	<i>L. interrogans</i> , <i>L. santarosai</i>
<i>szwajizak</i>	<i>L. interrogans</i> , <i>L. santarosai</i>
<i>valbuzzi</i>	<i>L. interrogans</i> , <i>L. kischneri</i>

**Table 8. Leptospiral serovars seen in multiple species (adapted from Bharti, 2003)**

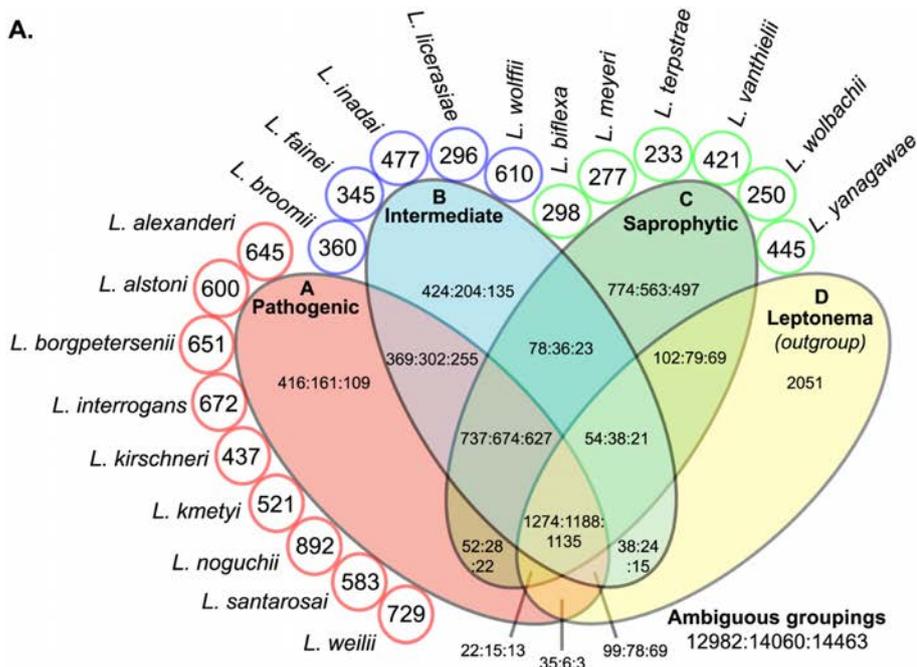
The reclassification of leptospire on genotypic grounds is taxonomically correct and provides a strong foundation for future classifications. However, the molecular classification is problematic for the clinical microbiologist, because it is clearly incompatible with the system of serogroups which has served clinicians and epidemiologists well for many years. Until simpler DNA-based identification methods are developed and validated, it will be necessary for clinical laboratories to retain the serological classification of pathogenic leptospire for the foreseeable future. In addition, the retention of *L. interrogans* and *L. biflexa* as specific names in the genomic classification also allows nomenclatural confusion. In the following pages, specific names refer to the genomospecies, including *L. interrogans sensu stricto* and *L. biflexa sensu stricto*.

In the 1990s, DNA hybridization (DDH) identified 17 ‘genomospecies’, which also distinguished DDH from serovar. DDH complemented by molecular methods and experimental studies have since confirmed the existence of at least 22 species, and grouping of species as infectious (sometimes referred to as group I and group II pathogens, corresponding to “pathogenic” and “intermediately pathogenic”, respectively) and non-infectious (“saprophytic”) (12). The International Committee on Systematics of Prokaryotes, Subcommittee on the Taxonomy of Leptospiraceae recently agreed that genome sequence comparison should replace DDH for species definition (19).

Leptospiral typing is important for carrying out outbreak investigations and in identifying likely mammalian host reservoir sources of infection. Two commonly used molecular methods performed are pulsed-field gel electrophoresis and multilocus sequencing typing (MLST). MLST

has the advantage that it reflects the underlying population genetic structure, is reproducible, is robustly supported by experimental data, and even can be used directly to identify infecting *Leptospira* in clinical samples (Agampodi, 2013). Genome sequencing, which has become widely available, together with automated tools that assign MLST sequence types directly from sequence data, has demonstrated an important potential for typing, with the expectation that automated analysis tools will become sufficiently user-friendly for rapid and efficient whole genome analysis and comparison, including phylogenetic analysis based on the identification of single nucleotide polymorphisms (SNPs) in the core genome.

The *Leptospira* Genome Project, initiated in 2011, has been to obtain and compare whole genome information for all known *Leptospira* species. Among the goals of this analysis were the following: i) identifying *Leptospira* pathogenesis mechanisms that might explain heterogeneity in clinical manifestations of leptospirosis; ii) understanding the relationship of genomic content and context to pathogenesis; iii) determining the definitive evolutionary relationship of *Leptospira* towards understanding how infectious *Leptospira* diverged from saprophytes; and iv) identifying common antigens for improving diagnosis and vaccine development. There are 9 known pathogenic *Leptospira* species, 5 intermediate *Leptospira* species, and 6 saprophytic *Leptospira* species for which the whole genome sequence analysis has been compared (Fouts, 2016) (Figure 4).

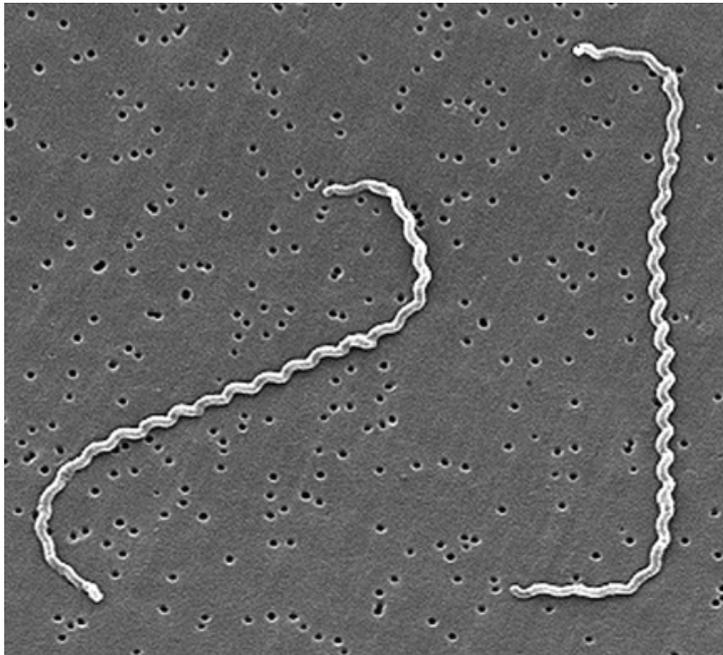


**Figure 4. Pan-genomic comparisons of 20 *Leptospira* species. Orthologous protein clusters were binned, counted and placed into a Venn diagram by whether clusters contained proteins from genomes in each of the three *Leptospira* groups: pathogenic (A), intermediate (B), saprophytic (C) and the *Leptonema* outgroup (D). Clusters were counted if there was a majority (50%), all-but-one, or all protein members from a particular group or groups (separated by colons). Singleton clusters, representing species-specific or strain-specific genes are noted in circles surrounding the Venn diagram. Clusters not matching any of these criteria or containing at least one protein from another group were considered as ambiguous groupings (adapted from Fouts, 2016).**

### II.3.3. Biology of leptospire

Leptospire are tightly coiled spirochetes, usually 0.1  $\mu\text{m}$  by 6 to 0.1 by 20  $\mu\text{m}$ , but occasional cultures may contain much longer cells. The helical amplitude is approximately 0.1 to 0.15  $\mu\text{m}$ , and the wavelength is approximately 0.5  $\mu\text{m}$ . The cells have pointed ends, either or both of which are usually bent into a distinctive hook (Figure 5). Two axial filaments (periplasmic flagella) with polar insertions are located in the periplasmic space. The structure of the flagellar proteins is complex. Leptospire exhibit two distinct forms of movement, translational and nontranslational. Morphologically all leptospire are indistinguishable, but the morphology of individual isolates varies with subculture in vitro and can be restored by passage in hamsters. Leptospire have a typical double membrane structure in common with other spirochetes, in which the cytoplasmic membrane and peptidoglycan cell wall are closely associated and are overlain by an outer membrane. Leptospiral lipopolysaccharide has a composition similar to that of other gram-negative bacteria, but has lower endotoxic activity. Leptospire may be stained using carbol

fuchsin counterstain. Leptospires are obligate aerobes with an optimum growth temperature of 28 to 30°C. They produce both catalase and oxidase. They grow in simple media enriched with vitamins (vitamins B<sub>2</sub> and B<sub>12</sub> are growth factors), long-chain fatty acids, and ammonium salts. Long-chain fatty acids are utilized as the sole carbon source and are metabolized by  $\beta$ -oxidation.



**Figure 5. Scanning electron micrograph of *L. interrogans* serovar icterohaemorrhagiae strain RGA bound to a 0.2- $\mu$ m membrane filter (adapted from Levett, 2001).**

#### **II.3.4. Immunization**

Immunity to leptospirosis is largely humoral and is relatively serovar specific. Thus, immunization protects against disease caused by the homologous serovar or antigenically similar serovars only. Vaccines must therefore contain serovars representative of those present in the population to be immunized. Immunization has been widely used for many years as a means of inducing immunity in animals and humans, with limited success. Early vaccines were composed of suspensions of killed leptospire cultures in serum-containing medium, and side effects were common. Modern vaccines prepared using protein-free medium are generally without such adverse effects. In developed countries, pigs and cattle are widely immunized, as are domestic dogs, but in most developing countries, vaccines which contain the locally relevant serovars are not available. Most vaccines require booster doses at yearly intervals.

Most bovine and porcine vaccines contain serovars hardjo and pomona. Protection against hardjo infection has been suboptimal, but one vaccine has been shown to offer good protection (Zuerner,

1995) and induces a cell-mediated immune response. Canine vaccines generally contain serovars *canicola* and *icterohaemorrhagiae*. Vaccines protect against disease and renal shedding under experimental conditions, but transmission of serovar *icterohaemorrhagiae* from immunized dogs to humans has been reported (Feigin, 1973). Moreover, immunized dogs may be infected with serovars other than those contained in commercial vaccines. A vaccine has been released recently which includes serovars *grippityphosa* and *pomona* in addition to the traditional vaccine strains, in response to the increasing incidence of canine infection with these serovars.

Human vaccines have not been applied widely in Western countries. Immunization with polyvalent vaccines has been practiced in the Far East, where large numbers of cases occur in ricefield workers, such as in China and Japan. In France, a monovalent vaccine containing only serovar *ictero-haemorrhagiae* is licensed for human use. A vaccine containing serovars *canicola*, *icterohaemorrhagiae*, and *pomona* has been developed recently in Cuba.

## **II.4. Pathogenesis**

The mechanisms by which leptospire cause disease are not well understood. A number of putative virulence factors have been suggested, including toxin production, attachment, and immune mechanisms. However, their respective role in pathogenesis remains unclear. Infectious *Leptospira* show key adaptations to mammals, for example sialic acid biosynthesis, pathogen-specific porphyrin metabolism, and the observation that pathogenic *Leptospira* are vitamin B12 autotrophs, able to synthesize it from a simple amino acid precursor, L-glutamine. A large novel protein family of unknown function—the Virulence Modifying proteins—is found uniquely in pathogenic *Leptospira*. Similarly, the CRISPR/Cas system is only found in pathogenic *Leptospira*.

### **II.4.1. Toxin production**

Endotoxic activity has been reported in several serovars (De Souza, 1992). Leptospiral lipopolysaccharide (LPS) preparations exhibit activity in biological assays for endotoxin, but at much lower potencies.

Serovar *pomona* is notable for the production of hemolytic disease in cattle, while serovar *ballum* produces similar symptoms in hamsters. Hemolysins from several serovars have been characterized. The hemolysins of serovars *ballum*, *hardjo*, *pomona*, and *tarassovi* are sphingomyelinases. Virulent strains exhibit chemotaxis towards hemoglobin. Plasma has been shown to prevent hemolysis. Phospholipase C activity has been reported in serovar *canicola*. A

hemolysin from serovar lai is not associated with sphingomyelinase or phospholipase activity and is thought to be a pore-forming protein.

Strains of serovars pomona and copenhageni elaborate a protein cytotoxin, and cytotoxic activity has been detected in the plasma of infected animals (Knight, 1973). In vivo, this toxin elicited a typical histopathologic effect, with infiltration of macrophages and polymorphonuclear cells. A glycolipoprotein fraction with cytotoxic activity was recovered from serovar copenhageni (Vinh, 1986).

#### **II.4.2. Attachment**

Leptospire have been shown to attach to epithelial cells. Virulent leptospire adhere to renal epithelial cells in vitro, and adhesion is enhanced by subagglutinating concentrations of homologous antibody. Leptospire are phagocytosed by macrophages (Cinco, 1981) in the presence of specific antibody. Phagocytosis occurs only in the presence of serum and complement (McGrath, 1984), suggesting that the outer envelope of leptospire possesses an antiphagocytic component. Leptospiral LPS stimulated adherence of neutrophils to endothelial cells and platelets, causing aggregation and suggesting a role in the development of thrombocytopenia (Isogai, 1997).

#### **II.4.3. Immune mechanisms**

The second stage of acute leptospirosis is also referred to as the immune phase, in which the disappearance of the organism from the bloodstream coincides with the appearance of antibodies. The clinical severity of the disease often appears to be out of proportion to the histopathological findings. Immune-mediated disease has been proposed as one factor influencing the severity of the symptoms.

Levels of circulating immune complexes were correlated with severity of symptoms (Galli, 1985), and in patients who survived, circulating immune complex levels fell concurrently with clinical improvement. However, in experimental infections in guinea pigs, leptospiral antigen localized in the kidney interstitium, while immunoglobulin G (IgG) and C3 were deposited in the glomeruli and in the walls of small blood vessels.

Antiplatelet antibodies have been demonstrated in human leptospirosis (Davenport, 1989). In leptospirosis and septicemia, such antibodies are directed against cryptantigens exposed on damaged platelets and do not play a causal role in the development of thrombocytopenia. Other

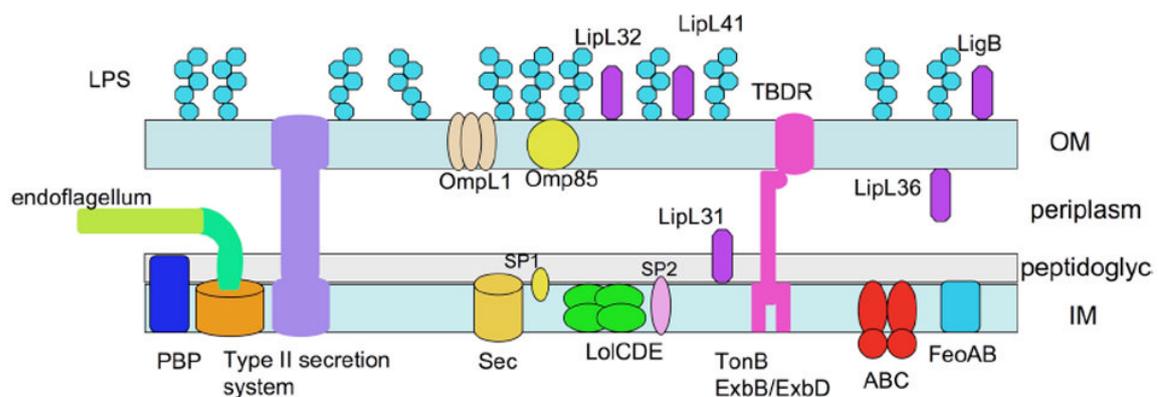
autoantibodies have been detected in acute illness, including IgG anticardiolipin antibodies and antineutrophil cytoplasmic antibodies. However, the significance of antineutrophil cytoplasmic antibodies in the pathogenesis of vascular injury in leptospirosis has been questioned (Abdulkader, 1993).

Virulent leptospire induce apoptosis in vivo and in vitro. In mice, apoptosis of lymphocytes is elicited by LPS via induction of tumor necrosis factor alpha (TNF- $\alpha$ ) (Isogai, 1998). Elevated levels of inflammatory cytokines such as TNF- $\alpha$  have been reported in patients with leptospirosis (Estavoyer, 1991)

#### II.4.4. Surface proteins

The outer membrane of leptospire contains LPS and several lipoproteins (outer membrane proteins (OMPs)) (Haake, 2000) (Figure 6). The LPS is highly immunogenic and is responsible for serovar specificity (Chapman, 1988).

*Leptospira* spp. possess a double-membrane structure. The peptidoglycan cell wall is associated with the inner membrane. The leptospiral outer membrane is known to contain the transmembrane porin OmpL1, and lipoproteins LipL32, LipL36 (at the inner leaflet of the outer membrane), LipL41, and LigB. *Leptospira* spp. possess a LPS which is composed of lipid A, a non-repeating oligosaccharide core and a distal polysaccharide (or O-antigen). As in other spirochaetes, the endoflagella is located in the periplasm. The inner membrane contains the penicillin-binding proteins (PBP), and the lipoprotein LipL31 (Chang, 2007).



**Figure 6. Schematic diagram of the cell wall of leptospire (adapted from Ko, 2009).**

#### **II.4.5. Immunity**

Immunity to leptospirosis is largely humoral in nature (Adler, 1977). Passive immunity can be conferred by antibodies alone (Adler, 1978). A serovar-specific antigen (F4) extracted from LPS lacked endotoxic activity and induced protective immunity in rabbits, guinea pigs, and mice (Faine, 1974). A similar antigen (TM), which inhibited agglutination by homologous antisera, was shown to be distinct from F4 but had a common epitope. Sodium dodecyl sulfate extracts of whole cells induced production of protective antibody, which was also agglutinating and complement fixing. Immunity is strongly restricted to the homologous serovar or closely related serovars.

Several of the leptospiral OMPs are highly conserved, and the potential for subunit vaccines which can generate broadly cross-protective immunity has been suggested by studies using OmpL1 and LipL41 (Hakke, 1999), which induced synergistic protection.

Cell-mediated immune responses to leptospirosis have been reported (Ratnam, 1984). However, suppression of the cell-mediated immune response has been reported (Yamashiro-Kanashiro, 1991), with reduction in the number of CD4+ lymphocytes and in their responsiveness to some mitogens.

#### **II.5. Bio-clinical features**

Leptospirosis ranges in severity from a mild, self-limited febrile illness to a fulminant life-threatening illness. When illness occurs, a broad array of organ systems may be involved, reflecting the systemic nature of the infection. As a result, the signs and symptoms of leptospirosis are protean and frequently mistaken for other causes of acute febrile syndrome.

##### **II.5.1. Incubation phase**

The incubation phase from exposure to onset of symptoms averages from 7 to 12 days, though it can be as short as 3 days or as long as a month. The remarkable variability in the duration of the incubation phase is evident in the 6–29 day lag between exposure and onset of symptoms among 52 athletes who developed laboratory-confirmed leptospirosis after participating (all on the same day) in the Springfield Triathlon (Morgan, 2002).

##### **II.5.2. Presentation**

The clinical presentation of leptospirosis is biphasic (Figure 7), with the acute or septicemic phase lasting about a week, followed by the immune phase, characterized by antibody production and

excretion of leptospire in the urine. Most of the complications of leptospirosis are associated with localization of leptospire within the tissues during the immune phase and thus occur during the second week of the illness. Patients typically present with sudden onset of fever, chills, and headache. These signs and symptoms are nonspecific and also occur with other causes of acute febrile syndrome that, depending on the setting, could also be caused by influenza, dengue fever, or malaria. The headache is often severe and has been described as a bitemporal, frontal throbbing headache accompanied by retro-orbital pain and photophobia. Muscle pain and tenderness is common and characteristically involves the calves and lower back. A tip-off to identification of leptospirosis is conjunctival suffusion (dilatation of conjunctival vessels without purulent exudate), which occurs frequently in leptospirosis, but is uncommon in other infectious diseases. Additional ocular findings typically include subconjunctival hemorrhages and icterus. Rash is uncommon in leptospirosis and when it occurs in the setting of an acute febrile illness, may suggest an alternative diagnosis such as dengue or chikungunya fever (Burt, 2012; Zaki, 2010). An erythematous rash limited to the pretibial areas of both legs appearing on about the fourth day of illness was a feature of an outbreak of “Fort Bragg fever” which also included headache, malaise, and splenomegaly among soldiers in North Carolina, the etiology of which was later determined to be *L. interrogans* serovar Autumnalis (Gochenour, 1952).

A nonproductive cough has been noted in 20–57 % of leptospirosis patients and can potentially lead clinicians to incorrectly diagnose the patient with influenza or another respiratory illness. Gastrointestinal symptoms are frequently observed, and may include nausea, vomiting, diarrhea, and abdominal pain. Nausea and other gastrointestinal symptoms may contribute to dehydration in patients with high-output nonoliguric renal failure caused by leptospirosis. The abdominal pain may be due to acalculous cholecystitis and/or pancreatitis. In patients admitted to the hospital for leptospirosis, abdominal pain associated with abnormal serum amylase and/or lipase levels is relatively common (O’Brien, 1998). It should be kept in mind that impaired renal function alone can elevate pancreatic enzyme levels when the creatinine clearance is less than 50 ml/min (Collen, 1990). While most cases of pancreatitis due to leptospirosis are self-limited, some cases are more severe and associated with fatal outcomes (Spichler, 2007).

Severe leptospirosis is characterized by dysfunction of multiple organs including the liver, kidneys, lungs, and brain. The combination of jaundice and renal failure, known as Weil’s disease, was first described in 1886 (Weil, 1886) and remains one of the most clinically recognizable forms of leptospirosis. Evidence of organ dysfunction indicates a more advanced stage of infection, yet

may develop suddenly and be present in a large percentage of patients at the time of presentation.

Leptospirosis patients are typically found to have mild to moderate elevations in levels of liver transaminases and direct (conjugated) bilirubin. The frequency of jaundice varies widely among case series, perhaps due in part to the virulence of the causative organism. One study reported a strong association between infection with the Icterohaemorrhagiae serogroup and jaundice and elevated bilirubin levels (Katz, 2001). Acute hemolytic anemia can contribute to jaundice which, not surprisingly, is more common in leptospirosis patients with glucose-6-phosphate dehydrogenase deficiency (Avdeeva, 2002). Such patients have a higher percentage of unconjugated (i.e., indirect) bilirubin. Many patients have leukocytosis and thrombocytopenia, though usually not to the extent that would cause spontaneous hemorrhage. Leukopenia in the setting of thrombocytopenia and anemia can suggest bone marrow suppression.

Clinical signs of bleeding are common and occur in the majority of patients with severe leptospirosis. Most bleeding manifestations are mild, including petechiae, ecchymoses, and epistaxis. However, some patients have severe gastrointestinal (melena or hematemesis) or pulmonary hemorrhage. Thrombocytopenia frequently occurs, although usually not to the extent that would cause spontaneous hemorrhage. In a study of severe leptospirosis performed in the Netherlands, all patients had coagulation disorders, including prolongation of the prothrombin time (PT) and the length of the PT was associated with severe bleeding manifestations (Wagenaar, 2010).

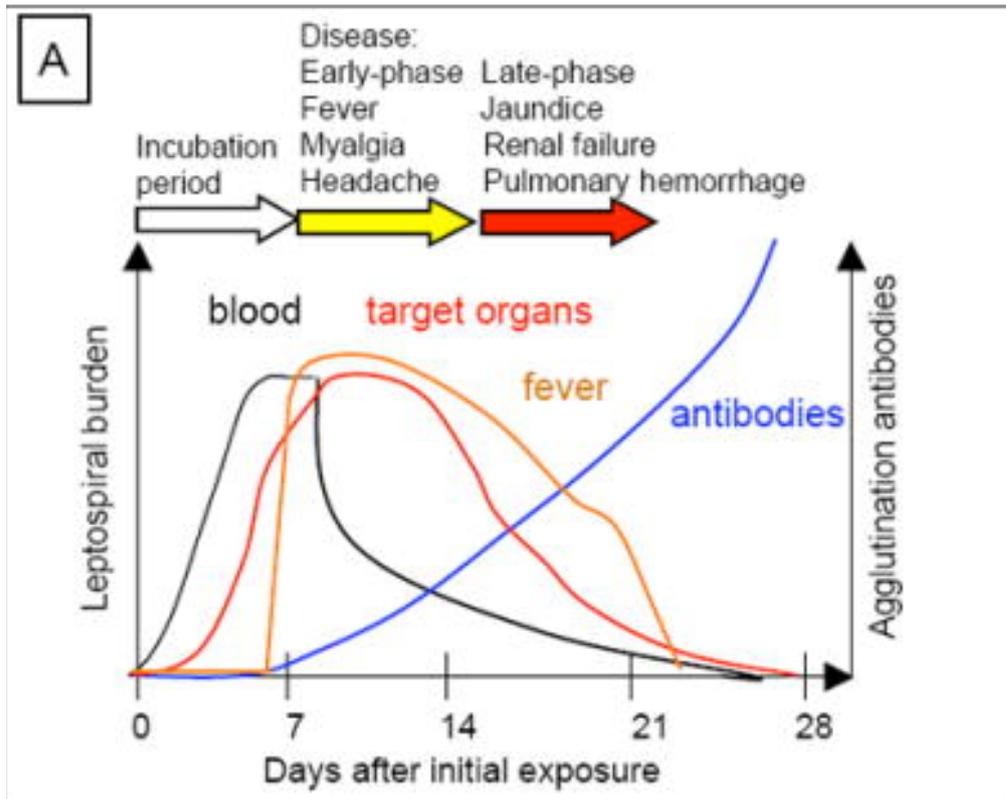
The kidney is a major target organ in leptospirosis, perhaps due to the intrinsic renal-tropic homing ability of leptospire in their reservoir hosts. The kidneys are commonly involved, as manifested by elevations in serum blood urea nitrogen and creatinine levels and findings on urinalysis of pyuria, hematuria, and elevated urine protein levels (Katz, 2001). Leptospirosis causes a unique nonoliguric potassium wasting nephropathy characterized by impaired sodium reabsorption and potassium wasting (Seguro, 1990). When poor oral intake due to nausea and high-output renal failure combine to cause dehydration, patients are at risk of oliguria and renal failure, a frequent cause of death in areas where peritoneal or hemodialysis **are** not available.

Progression to severe leptospirosis and circulatory collapse may be accompanied by acute respiratory distress syndrome (ARDS). As in other causes of ARDS, leptospirosis causes diffuse lung injury characterized by impaired gas exchange and the need for mechanical ventilation.

Massive hemoptysis, representing extensive alveolar hemorrhage, is an ominous complication of leptospirosis associated with fatality rates >50 % (Gouveia, 2008). Pulmonary hemorrhage associated with leptospirosis was first reported in Switzerland in 1943 (Moeschlin, 1943), and since then has been reported with increasing frequency from a variety of locations (Park, 1989). Leptospirosis-associated severe pulmonary hemorrhage syndrome (SPHS) can occur sporadically or in outbreaks that can be confused clinically with viral pneumonitis (Sehgal, 1995; Trevejo, 1998). For example, a 1995 outbreak of SPHS that occurred after heavy rainfall and flooding in Nicaragua was initially thought to be due to hantavirus pulmonary syndrome until silver staining and immunohistochemistry of postmortem lung tissue revealed leptospire (Trevejo, 1998). SPHS can present as hemoptysis associated with cough or may be discovered after patients undergo pulmonary intubation (Yersin, 2000). Chest radiographs show diffuse alveolar infiltrates. Epidemiologic evidence suggests that SPHS may be a relatively new problem, suggesting emergence of a new clone of *L. interrogans* with enhanced virulence. However, it is also possible that SPHS is an old problem that is finally being recognized and documented.

As noted above, headache is frequently severe and when accompanied by meningismus may prompt performance of lumbar puncture. Typical findings on CSF examination include a lymphocytic predominance with total cell counts of up to 500/mm<sup>3</sup>, protein levels between 50 and 100 mg/mL, and normal glucose levels, consistent with aseptic meningitis (Berman et al. 1973). Depending on the epidemiologic setting, leptospirosis may be a predominant cause of aseptic meningitis in some areas (Silva, 2002). Patients with aseptic meningitis due to leptospirosis may be anicteric, making the diagnosis more challenging (Berman, 1973; Karande, 2005). In severe leptospirosis, altered mental status may be an indicator of meningoencephalitis. A variety of other neurologic complications may also occur including hemiplegia, transverse myelitis, and Guillain–Barré syndrome (Levett, 2001).

The appealing hypothesis that distinct clinical syndromes were associated with specific serogroups has been refuted over the past 30 years. An explanation for many of the observed associations may be found in the ecology of the maintenance animal hosts in a geographic region. A region with a richly varied fauna will support a greater variety of serogroups than will a region with few animal hosts. In humans, severe leptospirosis is frequently but not invariably caused by serovars of the icterohaemorrhagiae serogroup. The specific serovars involved depend largely on the geographic location and the ecology of local maintenance hosts.



**Figure 7. Disease kinetics of leptospirosis. Schematic diagram of the kinetics of leptospiral infection and disease. Infection produces a leptospiraemia (black line) within the first days after exposure, which is followed by detection of leptospire in tissues of multiple organs (red line) by the 3rd day of infection. In humans, illness (fever, brown line) develops with the appearance of agglutinating antibodies 5-14 days after exposure (blue line). Leptospire are cleared from the bloodstream and organs as serum agglutinating antibodies titres increase. Although early-phase illness (yellow arrow) is mild and resolves in the majority of infected individuals, a subset of patients progress four to six days after the onset of illness to develop severe late-phase manifestations (red arrow) during the period of immune-mediated destruction and clearance of leptospire (black line) (adapted from Ko, 2009)**

### **II.5.3. Risk factors for morbidity and mortality**

In an active surveillance study of 326 cases of leptospirosis in Salvador, Brazil, the strongest independent predictor of a fatal outcome was altered mental status (odds ratio 9.12), which typically began with confusion and obtundation without focal neurologic signs (Ko, 1999). Other independent risk factors for death identified in the Salvador study included oliguria (odds ratio 5.28), age over 36 years (odds ratio 4.38), and respiratory insufficiency (2.56) (Ko, 1999). The risk of a fatal outcome increases with increased age; compared to individuals aged 19–29, the increased risk of death rose from 3.7-fold for 40–49 year olds to 7.3-fold among those 60 or older (Lopes, 2004). Lung involvement, as indicated by dyspnea (odds ratio 11.7) or alveolar infiltrates on chest X-ray (odds ratio 7.3), was found to be associated with mortality in a retrospective study

of 68 leptospirosis cases at Pointe-à-Pître Hospital in the French West Indies, along with oliguria (odds ratio 9), repolarization abnormalities on electrocardiogram (odds ratio 5.95), and a white blood count  $>12,900/\text{mm}^3$  (odds ratio 2.54) (Dupont, 1997). A retrospective review of leptospirosis cases associated with an outbreak of leptospirosis in India identified pulmonary involvement and altered mental status as independent predictors of death (Pappachan et al. 2004). Additional poor prognostic signs identified in other studies include acute renal failure, hypotension, and arrhythmias (Daher, 1999; Panaphut, 2002). Several risk factors for severity have been identified in New Caledonia, including cigarette smoking and late initiation of antibacterial therapy. *Leptospira interrogans* serogroup Icterohaemorrhagiae seems also to be linked to disease severity (Tubiana, 2013). First laboratory results concerning abnormal creatinine, lactates or amylase levels, platelet count and leptospiremia may alert clinicians on the potential severe evolution of the disease.

The abortive effect of this disease, well known in animals, is only reported rarely in humans although transplacental transmission has been demonstrated (Coghlan, 1969). A study performed in pregnant women in French Guiana showed that fetal death occurred in more than 50% of the cases due to premature abortion or in utero death. The rate of maternal-fetal transmissions is not known, but leptospirosis could be a not uncommon cause of perinatal deaths, premature delivery or low-weight birth (Chansamouth, 2016) in endemic zones.

#### **II.5.4. Recovery phase**

With proper supportive care, most leptospirosis patients recover completely (Spichler, 2011). Patients with acute renal failure who require dialysis typically regain most of their renal function, although there may be evidence of persistent mild renal impairment (Covic, 2003). In addition, there is growing recognition that many patients suffer from chronic post-leptospirosis symptoms. Leptospirosis may induce chronic symptoms analogous to those produced by other spirochete infections, such as Lyme disease, but the mechanisms are still unknown and the causal association has not been proven. However, no objective evidence supports or refutes this hypothesis. Specific biomarkers of acute and chronic manifestations of leptospirosis are not available other than specific serologies, PCR, and culture.

Late complications of leptospirosis include clinically observable disease such as uveitis (Rathinam, 2002) or chronic meningitis (Murgatroyd, 1937), and persistent abnormal laboratory markers such as serum creatinine (Daher, 2004; Ghasemian, 2016). A study reporting long-term follow-up

suggested that leptospirosis resolves in the vast majority of cases without chronic complications or sequelae (Spichler, 2011). In another study of laboratory-confirmed leptospirosis patients in the Netherlands, 30 % of patients experienced persistent complaints after acute leptospirosis characterized by fatigue, myalgia, malaise, headache, and weakness (Goris, 2013).

Ocular involvement in the form of uveitis is well-known to occur during the convalescent phase of leptospirosis. Eye involvement ranges in severity from insidious onset of mild anterior uveitis to acute, severe panuveitis involving the anterior, middle, and posterior segments of the eye (Rathinam, 2005). Leptospiral uveitis may occur either as a single, self-limited event or as a series of recurrent episodes, which appears to occur more frequently in patients with severe uveitis. In one study, 80 % of patients had leptospiral DNA in the aqueous humor, detected by PCR (Chu, 1998). However, the relative contributions of infection and autoimmunity are uncertain.

## **II.6. Control and surveillance**

The lack of an adequate diagnostic test remains a foremost barrier to implement effective policy and responses to leptospirosis. The distribution of the leptospirosis burden overlaps significantly with that for malaria, dengue, and enteric fever. Misdiagnosis between these diseases is common and in the case of leptospirosis, leads to delayed treatment of severe complications and poor outcomes. Development and roll-out of diagnostic protocols could be leveraged and implemented synergistically that aim to address the multiple causes of acute fever in resource-poor, high-burden regions.

### **II.6.1. Control (WHO, 2002)**

Because of the large number of serovars and infection sources and the wide differences in transmission conditions, the control of leptospirosis is complicated and will depend on the local conditions. Control can be achieved by controlling the reservoir or reducing infection in animal reservoir populations such as dogs or livestock. **Control of wild animals will be difficult.**

Preventive measures must be based on a knowledge of the groups at particular risk of infection and the local epidemiological factors. Prevention and control should be targeted at : (a) the infection source; (b) the route of transmission between the infection source and the human host; or (c) infection or disease in the human host. It is important to establish what animal species are the infection sources in a particular area. Control measures can then be targeted to the local reservoir species of animals. Such measures include: the reduction of certain animal reservoir populations, e.g. rats; the separation of animal reservoirs from human habitations by means of fences and

screens; the immunization of dogs and livestock; the removal of rubbish and keeping areas around human habitations clean; encouraging people not to leave food around, especially in recreational areas where rats may be present. It is important to be aware of the risk factors for human infection and, if possible, the infection source. Risk of infection is minimized by avoiding contact with animal urine, infected animals or an infected environment. Where appropriate, protective clothing should be worn and wounds covered with waterproof dressings to reduce the chance of infection if exposure is likely, e.g. occupational or recreational exposure.

### **Intervention at the source of infection**

Control can take place in the form of interventions at the source of infection (reservoir host/carrier/shedder), at the transmission route, or at the level of the human host. It should be remembered that many serovars and many reservoir hosts, each with their own special ecological niche, may occur in a given area. **In addition**, there are many ways in which humans can become infected, depending on the infection source and the prevailing environmental conditions. In view of the foregoing, no general rules for the control of leptospirosis can be laid down and, for each situation, specific solutions must be found. The eradication of leptospirosis in feral animals is not possible, but in small, defined populations (dogs, certified cattle herds, etc.) control measures can be highly effective (**Everard, 1979**). Exposure histories together with occupation and recreational pursuits may help to identify the infection source. If a probable infection source or sources have been identified, the suspected animals should be examined to determine whether they are carriers of leptospire and are shedding the organisms in their urine. Small animals can be trapped and identified, and after euthanasia, blood, urine and tissues (kidneys) can be aseptically removed for examination. Specimens from peridomestic animals can be collected on farms for leptospirosis examination (possibly in collaborative projects on other health problems in animals) or in slaughter-houses. Urine from large domestic animals can be collected after administration of a diuretic or by catheter. Blood obtained from animals should be examined by serology, and kidney tissue and urine by culture. Isolates from animals should be typed and compared with those from humans.

It is essential to be aware of the limitations of the methods used. Serology is of limited value as animals may be carriers without evidence of antibody production and the detection of antibodies does not imply carrier status. Culture is of limited value, as fastidious leptospire may fail to grow, and some animals may only shed organisms intermittently.

Possible intervention include the following: infected animals (cattle/pigs/dogs) can be isolated and if necessary slaughtered or killed; infected animals can be treated with antibiotics to control

leptospirosis; rodents (rats, mice) can be poisoned; rodents and other feral animals can be trapped; rodents and other feral sources of infection can be denied access to the human living environment by erecting fences, screens and rodent-proof buildings and stables; rodents (rats, mice) can be denied access to food and drinking-water, by building rodent-proof warehouses and other food/harvest depots, water reservoirs, stables, yards and pens, and by moving all spilled and waste food out of reach of pests; rodents and other feral animals can be deterred from living in areas of human habitation by keeping the surroundings scrupulously clean, removing rubble and litter, cutting down tall grass and shrubs, and installing adequate sanitation, and in particular waste disposal with good sewers and toilets, and by providing clean water; vaccines can be used for the immunization of pets and farm animals: e.g. those used for dogs may contain as immunogens the serovars icterohaemorrhagiae and canicola; for cattle: hardjo and pomona; for pigs: pomona, tarassovi and Bratislava; excreta from domestic animals should be disposed of in such a way as to avoid contamination.

#### **Intervention at the transmission route**

Transmission can be prevented by wearing protective clothing (boots, gloves, spectacles, aprons, masks); covering skin lesions with waterproof dressings; washing or showering after exposure to urine splashes or contaminated soil or water; washing and cleaning wounds; developing an awareness of potential risks and methods of preventing or minimizing exposure, e.g. by avoiding or preventing urine splashes and aerosols, avoiding touching ill or dead animals, fetuses, placentas, organs (kidneys, bladders) with bare hands, and, unless wearing gloves, avoiding assisting animals giving birth; wearing gloves when handling the urine of dogs and other animals, washing hands afterwards, and being aware that it is possible to be infected while nursing sick dogs or other animals; strictly maintaining hygienic measures during care or handling all animals and avoiding contact with urine or other body fluids; where feasible, disinfecting contaminated areas (scrubbing floors in stables, butcheries, abattoirs, etc.); providing clean drinking-water; preventing access to, or giving adequate warning of water bodies known or suspected to be contaminated (pools, ponds, rivers); mechanizing risk activities such as harvesting rice or cutting cane; introducing good herd management (avoiding communal pastures, buying certified leptospire-free stock); laying down and introducing standard safety procedures in laboratories.

#### **Intervention at the level of the human host**

Raising awareness is an important approach in both the general population and risk groups. People need to understand the disease and, if possible, how to avoid risks, but also that timely medical

help should be sought if leptospirosis is suspected after exposure. Clinicians and veterinarians should consider leptospirosis as part of the differential diagnosis in appropriate cases and provide treatment in an appropriate and timely fashion, while the public health and general authorities should introduce preventive measures. A full prophylactic course of treatment is required if exposure is known to have occurred, e.g. as a result of a laboratory accident or other high-risk exposure. Doxycycline is reported to give some degree of protection to exposed individuals from non-endemic areas (Sehgal, 2000). However, even if it does not always prevent infection, it can reduce the severity of the disease and thus mortality and morbidity. In countries where vaccines are available, immunization can be considered when there is a significant public health problem. Vaccines give protection only against the serovar, or at best the serogroup, present in the vaccine components so that vaccines combining several antigens may be needed. Protection is short-lived and boosting is needed. Vaccines may produce side-effects and are available only in certain countries.

### **Educational methods**

Information on the symptoms of leptospirosis, risk factors, diagnostic testing and therapeutic strategies should be regularly disseminated to licensed physicians and health care providers. Direct mailings, articles in the health department/public health agency's newsletter/report, articles in the local medical journal(s), grand round presentations at hospitals and ambulatory care facilities or, best of all, a combination of all these can be used for this purpose. Maintaining a high level of suspicion in the minds of clinicians will greatly enhance case identification. The successful impact of an active physician education programme can be readily seen by comparing the percentage of correct initial clinical impressions of physicians in Hawaii (62%) (Katz, 2001) with those of physicians in the USA in general (27%) (Martone, 1979) when confronted with an eventually confirmed case of leptospirosis.

Widespread community education can greatly assist in the identification of risk factors, the prevention of illness, and reducing the duration of illness and its severity through the early recognition of suspicious symptoms and self-referral for evaluation and treatment. Various methods can be used, as discussed below. Brochures can be produced inexpensively and made widely available and distributed to clinics, health departments, agriculture departments, the military, etc. They should describe the disease, how it is recognized clinically, how it is treated and methods of preventing exposure. The brochure should be written in the languages most frequently used in the community. Contact telephone numbers should also be given for those who want more

information. If politically acceptable, brightly coloured warning signs can be used to attract people's attention. Such signs may list one or two primary risk factors, together with a contact department and telephone number for more information. These may be placed at sites where there is a known risk of exposure, as well as at locations where people at risk will be likely to see them. If resources permit, a descriptive videotape can be produced by the health department or by an ad hoc committee, describing the disease and how it is recognized, treated and prevented. Videotapes should be distributed in district health offices, libraries and schools, and be available for free public loan at videotape rental stores. Information on their availability should be widely publicized. Display boards with tables, graphs, and photographs of the disease, together with information on its recognition, risk factors, treatment and prevention, are inexpensive to make and may be very helpful in promoting better understanding in health fairs, hospitals, clinics, schools and libraries.

The ad hoc committee should consider having tee-shirts produced with a suitable message on the reverse. They can be worn during any educational activity and given to anyone who wants one and is willing to pay the cost of production. Educational signs can be developed by the ad hoc committee to be placed in buses to increase awareness of the disease and the methods for its prevention. A contact telephone number should be given on the sign to enable interested people to obtain more detailed information.

When an outbreak occurs (e.g. after a hurricane or flooding), both physicians and the general public need to be rapidly informed of the situation and of the ways in which the disease may be prevented. Through press releases and mailings, physicians should be provided with information to help them to recognize a febrile illness possibly due to leptospirosis, and on suitable treatments for the disease. Again through press releases and radio and television announcements, and possibly also by putting up signs if the outbreak is confined to a specific location, information should be provided to the general public on the clinical signs of leptospirosis, on the risk of exposure, and on the importance of seeing a physician as soon as possible because the disease responds to antibiotics. Information should also be given on methods of prevention, e.g. warning people not to drink or submerge themselves in water that may be contaminated, and not to wash clothes in potentially contaminated water if any skin lesions are present. People should also be informed of the risks to animals of a large-scale outbreak, e.g. one resulting from flooding associated with a hurricane. Signs and symptoms seen in animals should be publicized as well as the vaccines that, if available, can be administered to animals concurrently with an antibiotic (e.g. penicillin) to minimize the risk that they will develop infections.

Immunization by means of vaccines seems to provide a certain degree of protection. Vaccines are, in principle, suspensions of killed leptospire. Protection is largely serovar-specific. In areas where many serovars are causing leptospirosis, a vaccine must consist of different serovars matching those circulating locally. In some countries, e.g. China, where many serovars occur, vaccines consist of a mixture of a few of the most prevalent. Protective antibodies are produced only against the serovars present in the particular vaccine used.

Information on human vaccines is limited, and they are available only in certain countries. Vaccines have been reported to give some degree of protection, and this is particularly important in areas where more serious forms of leptospirosis occur and where access to medical services is limited or delay in receiving treatment is likely. However, protection is of relatively short duration, and boosting at regular intervals is necessary to maintain protective titres of antibodies. Vaccines may also produce side-effects, such as pain at the injection site, and fever. Animals can be immunized with vaccines consisting of suspensions of killed leptospire. Protection is largely serovar-specific. Immunization may prevent disease but does not always prevent the development of renal carriage. Small areas, such as floors, can be cleaned and disinfected, but disinfecting large natural areas such as lakes or rivers is not possible. Leptospire are sensitive to many environmental influences. They are rapidly killed by disinfectants and desiccation. However, leptospire shed in animal urine can survive in the environment for weeks to months under suitable conditions, e.g. in moist soil or surface water with a neutral or slightly alkaline pH.

### **II.6.2. Surveillance and outbreak management**

In view of the complexity and variability of the epidemiology of leptospirosis in its different manifestations, reliable baseline data as revealed by surveillance are needed before it is even possible to consider starting a control programme. Leptospirosis occurs worldwide but reliable data on its incidence and prevalence in different areas are scarce. It is probably overlooked and underreported in many parts of the world (Cassadou, 2016).

#### **Hospital-based studies**

The diagnosis should be confirmed by laboratory tests as the clinical manifestations of leptospirosis are often untypical. Leptospirosis should be suspected in patients presenting with symptoms such as fever, severe headache, prostration, aching muscles or conjunctival suffusion, or patients presenting with signs of aseptic meningitis, adult respiratory distress syndrome with

pulmonary haemorrhage, kidney failure or jaundice. Data should be collected on the age, sex, occupation and exposure history (place, date, conditions of animal contact or contact with contaminated environment) of the patient.

Laboratory methods should include reliable serology and culture which, even if it does not contribute to early diagnosis, confirms it when leptospires are isolated. Isolation followed by typing is essential for surveillance as it provides information about the leptospires circulating in a certain area. In addition, typing data can be compared with the clinical manifestations of the disease in the area concerned. Serology is also important but, because of cross- reactions, the information obtained is of limited value in terms of causative serovars. Mild cases may not be admitted to hospital, so hospital-based studies may result in a bias towards severity in assessing the public health importance of leptospirosis.

Very severe cases may also be missed as patients may die at an early stage of the disease before the diagnosis can be established. Especially in these cases, culture, PCR and immunohistochemistry may be useful methods of demonstrating the leptospiral etiology in postmortem samples.

### **Serosurveillance**

The detection of persisting antibodies by the MAT may give an indication of the prevalence of leptospirosis in an area. Serosurveillance provides data on leptospirosis as an infection rather than as a disease, and thus as a public health problem, since seropositivity may also be found in very mild cases. It is best carried out with the MAT using a panel of antigens that is representative of the locally circulating leptospires. If the local strains are not fully known, a broad panel with representative strains of all currently known serogroups should be used. Persisting antibodies from a past infection are usually serogroup-specific. Titres to serovars used as antigens and their frequency of distribution may give information on the prevalence of these serovars or on antigenically similar serovars belonging to the same serogroup. ELISA tests provide information only on recent or current cases and no information on the circulating serovars because they use a broadly reactive so-called genus-specific antigen to check for IgM antibodies.

### **Risk groups**

Studies may also be performed on selected groups (rice farmers, meat workers, etc.) in a population that is likely to be exposed to leptospires. While the incidence rate for the whole of the

population in an area may be low, it may be very high in a selected risk group. Preventive methods may be focused on a risk group.

### **Cohort studies**

Cohort studies in which the same risk group in the population is re-examined after an interval may, through seroconversion, provide information on the incidence of infection. Questionnaires on signs and symptoms that occurred during the interval may give an indication of the incidence and presentation of leptospirosis as a disease.

### **Active surveillance and case-control studies**

Active surveillance is helpful in determining the incidence of leptospirosis in a community (Sasaki, 1993). Physicians should be asked to obtain whole blood and serum for culture and serology from consenting patients presenting with clinical symptoms that meet a case definition established by the US Centers for Disease Control and Prevention, WHO, the Ministry of Public Health or the national government. Questionnaires should be administered designed to characterize the clinical illness and exposure history. Follow-up serum should be requested, e.g. 2–3 weeks after the first sample is taken for serological confirmation or earlier, since seroconversion may occur as early as 5–7 days after the onset of the disease. Such active surveillance may provide valuable information on the "normal" incidence of leptospirosis in a community and may identify serovars present in the area. Networks of public health laboratories may enable samples to be transported to a central laboratory.

Together with active surveillance, a concurrent case-control study to evaluate risk factors may identify previously unknown risks of exposure to leptospirosis. In addition to the information collected above and appropriate diagnostic samples, a second questionnaire covering all the relevant risk factors should be administered. The controls for this study may be those surveyed and found not to have the disease. Possible risk factors (during the 4 weeks before the onset of illness) include exposure to various animals and their urine, exposure to fresh water or mud, and specific activities involved in the exposures, e.g. swimming, feeding pigs and cleaning pig pens, the presence of skin wounds, drinking surface or catchment water, and walking barefoot.

### **Laboratory support**

Where health care is severely limited in terms of facilities and resources, investigation of leptospirosis may be restricted to simple serological screening methods. If laboratory facilities and

financial resources permit, the development and/or introduction of an IgM-ELISA or a similar test using broadly reactive antigen (genus-specific) for the serodiagnosis of different forms of leptospirosis is advised. Test kits may be purchased from various commercial sources or developed "in house". If resources are available and there is national support for setting up a reference/referral laboratory, the MAT should also be added to the diagnostic repertoire. A genus-specific test (e.g. ELISA) in combination with the MAT usually enables infection to be confirmed and the infecting serogroup to be determined by the MAT, although only rarely will it be possible to determine the serovar. In addition to offering serological confirmation and diagnosis, the culture of leptospires could also be carried out since facilities for this purpose will be needed to maintain strains for use in the MAT. A validated PCR may be used in well-equipped laboratories with experienced staff.

### **Community diagnostic services**

For diagnostic services, four levels of diagnostic capability are envisaged, as follows:

- First level: this is the most peripheral level of health care. Simple screening methods for anti-*Leptospira* antibodies should be available.
- Second level: moderately complicated serological methods and perhaps cultures can be handled and the observations made at the first level can be checked.
- Third level: this can handle all the complicated diagnostic methods, check the findings at the second level and perform quality control. For public health purposes, provisional typing of isolated leptospires can also be carried out.
- Fourth level: this is the level of national or international reference laboratories or other expert centres that maintain culture collections, perform typing and check the quality of performance in other laboratories.

Laboratories that can perform epidemiological studies, e.g. on infection sources and modes of transmission, are particularly valuable in surveillance and in the introduction and evaluation of control measures. However, some limitations of current diagnostic must be considered, including the lack of accurate rapid diagnostic tests and the lack of accessible external quality control systems for MAT. In addition, few labs in endemic areas are equipped with MAT. Finally, the lack of standardization of PCRs and variability of the specificity for intermediate group suggest results should be cautiously interpreted.

### **Public health impact**

If there is no reliable notification system, public health authorities suspecting that leptospirosis

occurs may investigate the occurrence of the disease and its impact on human health by collecting clinical samples, especially serum, from hospital patients suffering from a disease that fits the clinical description of leptospirosis. These samples should be examined in a laboratory offering leptospiral testing. In this way, information will be obtained on the occurrence of severe leptospirosis. Mild cases may be missed, however, since they are unlikely to be admitted to hospitals. Physicians should therefore be invited to submit clinical samples from patients in the community suspected of having leptospirosis, and these samples should be examined in a competent laboratory. The samples should include paired serum samples and whole blood (heparinized) for culture. Alternatively, blood samples can also be collected from known risk groups and examined for leptospiral antibodies. The members of such risk groups should be questioned about illness in the past that might have been leptospirosis. Finally, random blood samples may be examined to determine the seroprevalence. Random blood samples can also be taken from the general healthy population and tested for antibodies. However, such sampling would be expected to be the least useful of the methods previously mentioned. Thus, in a study in Hawaii in a high incidence area, samples were taken from several hundred blood donors but very few detectable titres were found; the overall prevalence was approximately 0.5% (Sasaki, 1993). Serological assessment of hospitalized or ambulatory patients with febrile illnesses would be much more likely to provide clues to the incidence of unrecognized leptospirosis, while taking blood samples from persons belonging to known high-risk groups will be helpful in assessing or confirming endemic levels of infection. Such seroprevalence studies may reflect exposure but not necessarily overt disease.

### **Animal surveillance**

Surveillance of feral and domestic animals may be necessary to complement surveillance among humans in looking for infection sources. Animal sources can be identified by serological testing and by culture of tissues and/or urine. Serological testing can provide rapid results, but may give only limited information on infection rates and circulating serovars. Animals may carry leptospires without having detectable antibodies (Weekes, 1997). Cultures are subject to contamination and require a prolonged incubation period of up to 6 months. However, culture can provide definitive identification of the infecting serovar. Different animal species are affected differently by the disease. Rodents usually do not show signs of illness, but once infected, shed the organisms in their urine for their lifetime (Taylor, 1991). The disease has different clinical patterns in dogs (Everard, 1979), cattle (Miller, 1991) and swine (Collares-Pereira, 1991), which are considered the primary domestic animal reservoirs that may transmit the disease to humans, although horses and

other animals may be clinically affected (Minette, 1983). Animal surveys are helpful in determining the primary reservoirs in a community, the extent of infection in the species, and the possible geographical variations in infection rates by species. Most screening tests for leptospirosis have not been thoroughly evaluated in animals and some, e.g. ELISA tests, require conjugates developed from the animal species being tested, although the latex agglutination and indirect haemagglutination tests developed for human testing may be used (Chappel, 1992). The preferred test is the MAT, if available, which may also provide some indication of the serogroups/serovars causing infection. However, serological sampling is inappropriate in rodents because it underestimates infection since many infected animals do not show an antibody response. Maximizing the sensitivity of diagnosis in rodents requires both kidney culture and MAT serology. Culturing of kidney tissues from euthanized animals is the most reliable method of detecting infection in animals. However, the kidney samples must be aseptically extracted from the animal, macerated or cut into small sections with sterile instruments, and inoculated into culture media containing 5-fluorouracil or appropriate antibiotics that suppress the growth of bacterial contaminants while leaving leptospire unaffected.

Rodent surveys require live-trapped animals, while those of swine and cattle may be conducted at abattoirs after the animals are killed and the abdominal cavity opened during evisceration. Culture of urine from live domestic animals may be undertaken, although it is difficult and samples easily become contaminated. The animal may be given a diuretic to increase urine production. The genitals can be washed with a disinfectant solution (chlorhexidene), and should then be wiped with a clean, dry cloth. A midstream urine sample will prevent mixing, and should be collected in a sterile container. The sample should be processed within 2 hours, centrifuged, and the pellet inoculated into culture media that suppress the growth of bacterial contaminants as described above for tissue samples.

### **Checking the environment for leptospire**

Samples of water and soil can be cultured and checked for growth of pathogenic leptospire (Saito, 2013). However, such tests may take several weeks or even months to complete and the results are therefore retrospective and apply only to the time and place at which the samples were obtained. PCR can be attempted, but the same disadvantages apply. Experimental animals have also been used as sentinels. The sensitivity of environmental sampling is poor. It is important to be aware that saprophytic leptospire can live in the environments, where pathogenic leptospire can be shed by animals, and thus can interfere with the detection of pathogenic leptospire.

### **Likelihood of leptospiral contamination**

Proof of the occurrence of pathogenic leptospires by culture and typing is, in principle, epidemiologically relevant. However, it may take weeks to months to obtain results as leptospires grow slowly. Negative results do not exclude the presence of pathogenic leptospires as they may be present in the environment but unevenly distributed. They may therefore not be present in the small sample examined. In addition, the environment may have been free of pathogenic leptospires when the sample was taken but may have been contaminated soon afterwards by an infected animal.

### **Safety of the environment**

If it is possible that infected animals are present and have access to surface waters, it is logical to assume that the environment will be regularly contaminated with pathogenic leptospires and that there is a risk of infection. It is therefore impossible to state categorically that surface water is safe because it is not possible to collect and examine a water sample that is representative of a large body of water. Finding pathogenic leptospires in the water by culture, or perhaps by PCR or another suitable method, is proof of their occurrence in the water at the time and place of sampling and confirms that there is a risk of infection. However, failure to find them does not necessarily mean that they are absent. Whether the presence of pathogenic leptospires results in infection or not will depend on a number of factors such as the concentration of leptospires, the duration of exposure, the possibility that leptospires can penetrate the human body, etc. If contamination of surface waters with pathogenic leptospires is suspected, a possible way of assessing the likelihood of contamination is to check the area around the water body for the presence of animals that may act as infection sources. This advice applies to all situations where transmission is suspected.

### **Endemic leptospirosis**

In many health departments, one person or a small group of people will be responsible for the surveillance and control of leptospirosis together with other diseases. When leptospirosis is a major problem in an area, it is helpful to form an ad hoc committee to assist in developing control measures in the community. Such a committee, usually headed by a public health professional, may include individuals representing various disciplines that are relevant to the problem. These will include specialists in public health disease surveillance, public health laboratory staff, rodent-control experts, representatives from the local college or university (public health epidemiologists, specialists in infectious diseases, preventive medicine, environmental health and safety, and

veterinarians), practising physicians, beef and dairy cattle farmers, pig farmers, officials responsible for local parks and recreation, fisheries and wildlife, and agriculture, and military officers concerned with preventive medicine. Together, a committee can be more effective in planning and implementing effective control and prevention measures in the community and in identifying resources that may increase the scope of community education.

### **Outbreak of a disease resembling leptospirosis**

Leptospirosis is easily confused with other febrile illnesses. Weather conditions leading to flooding may increase the risk of leptospirosis and other diseases. An early response to outbreaks of unexpected febrile illness should include rapid screening tests for leptospirosis.

In contrast to most viral infections, an effective and specific treatment is available for leptospirosis. Therefore, where possible, attempts to diagnose leptospirosis should be encouraged so that prompt treatment with suitable antibiotics and other supportive measures can be provided. This requires the collection of clinical samples for laboratory testing. For outbreaks in remote areas, local use of screening tests to detect antibody can be helpful. However, the limitations of such tests must be recognized and, in particular, the fact that antibodies are not detectable until at least 5–6 days after the onset of symptoms of disease. To detect animal reservoirs, domestic and wild animals should be investigated for infection with leptospire by serology and culture. The findings should be matched with findings in leptospirosis patients. When an outbreak of leptospirosis is suspected or identified by diagnostic tests, appropriate information should be provided to those at risk. This may include press releases (providing information on the identification of the disease, the importance of seeking appropriate medical attention for those who develop illness, and ways that exposure may be limited or prevented), and posting of warning signs if the outbreak is confined to a few sources (e.g. lakes).

### **II.7. Treatment**

There remains some controversy about whether antimicrobial treatment of severe leptospirosis should even be initiated since most cases of acute leptospirosis resolve spontaneously (Vinetz, 2003). In a prospective, randomised controlled trial of 79 patients (38 treated with penicillin G and 41 untreated patients), there were no differences with respect to time required for normalisation of biochemical parameters, duration of fever, or mortality (Edwards, 1988). A Cochrane database review that assessed antibiotic effectiveness in leptospirosis concluded that, because of a small number of published randomised clinical trials, there was insufficient evidence to provide clear guidelines for practice (Brett-Major, 2012). However, a recent systematic review explored

mortality of untreated leptospirosis showing that mortality was significantly higher in older patients with icteric disease or renal failure, but was lower in younger, anicteric patients (Taylor, 2015). Empirical treatment strategies should therefore prioritize high-risk individuals. In addition, several case series have reported shortened duration of illness when appropriate antibiotic therapy was administered during the initial phase of the illness (within 2–4 days). In patients with severe disease, late administration of antibiotics has also shown clinical efficacy and reduction in mortality rates (Taylor, 2015). Therefore, treatment of leptospirosis patients continues to be supportive management and use of appropriate antibiotics. Most experts would not withhold antimicrobial treatment when clinical findings and epidemiological exposure history suggest leptospirosis. Suggestive evidence supported the use of penicillin and doxycycline (Watt, 1988). Leptospire are sensitive in vitro to most antimicrobial agents, but the relevance of the in-vitro findings to clinical outcome for these agents has not been assessed in clinical trials. Currently recommended regimens and dosages are based on the severity of the disease. Doxycycline or amoxicillin are recommended in mild disease, whereas penicillin G and ampicillin are indicated for severe disease (Vintez, 2003).

## **II.8. Jarisch-Herxheimer reaction**

The knowledge of the Jarisch-Herxheimer reaction (JHR) dates back to the 15th century, when arsenical ointment was used to treat the “great pox” (syphilis). The initial literary description was given by Adolf Jarisch (Austrian dermatologist) in 1895, when he noted exacerbation of lesions in roseolar syphilis patients after treatment with mercury (Jarisch, 1895). Subsequently, in 1902, Karl Herxheimer (German dermatologist) independently reported a similar phenomenon (Herxheimer, 1902).

The JHR is defined as a transient immunological reaction, classically seen in patients during antimicrobial treatment (Bryceson, 1976). Clinically, it manifests within 24 h with short-term constitutional symptoms (fever, chills, headache, myalgias). Since its initial description in syphilis, the reaction has been noted in other spirochaetal infections, including tick-borne relapsing fever (Dworkin, 1998), Lyme disease (Maloy, 1998), and louse-borne relapsing fever (Butler, 1978). However, a clear idea of importance of JHR in leptospirosis is lacking.

### **II.8.1. Physiopathological considerations**

Despite advances in medicine, the exact mechanism of JHR remains obscure. Most of our understanding stems from research done in spirochaetal infections in general. Over the years, many

theories have been previously advanced to describe its pathophysiology. Herxheimer postulated that the liberation of an endotoxin from degenerating treponemes following treatment of syphilis was the underlying mechanism. These products react with the “sensitized” syphilitic tissues and cause aggravation of lesions (Herxheimer, 1925; Gelfand, 1976).

Lipopolysaccharides are biologically active constituents of bacterial cell membranes, which are released spontaneously upon exposure to various triggering factors (e.g. antibiotics, such as cefotaxime and tetracycline) (Evans, 1993) and can be accompanied by an inflammatory response. The choice of antibiotics used for the treatment of spirochaetal infections was also thought to affect the incidence and severity of the reaction, although studies in this regard have expressed conflicting views (Butler, 1978; Gebrehiwot, 1992; Nadelman, 1992; Pound, 2005). At present, there is no clear evidence suggesting the role of a particular antibiotic class on the occurrence of the JHR.

It has been proposed that the reaction follows an “all or none” principle i.e. a “certain number” (unknown) of organisms must be present for it to occur (Moore, 1948; Pound, 2005). The release of an endotoxin-like material (lipoprotein) from spirochaetes and the elevation of cytokines following antibiotic treatment, has received much consideration. While the exact sequence of events is not clear, the probable inducer of this reaction is thought to be the pro-inflammatory lipoproteins which are released from dead/dying organisms following antibiotic treatment. This leads to massive phagocytosis by mononuclear cells which results in the release and transient elevation of pyrogenic cytokines, such as TNF- $\alpha$  (first to rise), IL-6 (rises at the onset of symptoms) and IL-8 (last to rise) in different stages of JHR, thus causing the different symptoms of the reaction (Friedland, 1991; Negussie, 1992; Radolf, 1995; Radolf, 1991). This is further corroborated by the favorable response of JHR to corticosteroids which are known to cause transcriptional down-regulation of TNF, IFN and IL-6 (Boumpas, 1991). In recent years, the role of circulating immune complexes (CIC) in the development of the reaction has gained more emphasis. Initial reports were equivocal in detecting CICs, however experiments conducted later have shown a fourfold (or more) rise in the levels of IgG and IgM containing CICs triggered by antigen release from spirochetes, between two and eight hours after the initial therapy in patients with secondary syphilis experiencing a reaction (Loveday, 1993). **There is no published evidence of Jarisch-Herxheimer based on valid biological markers referring to leptospirosis.**

### **II.8.2. Clinical and biological characteristics of JHR**

The reaction occurs within a few hours (2-8 h) after the administration of the first dose of an

appropriate antibiotic, and usually resolves even without any intervention, in 24 h. The JHR is more severe in situations where the number of organisms is abundant and is characterized by a triad of transient events (fluctuation in body temperature, flare, physiological changes) (Colben, 1999). Fluctuation in body temperature is accompanied by flu-like symptoms including malaise, headache and chills, pharyngitis, tender lymphadenopathy and leucocytosis. This is followed by sweating and subsidence of fever. Physiological changes include hyperventilation, vasoconstriction and a rise in blood pressure, which occur in the early phases of the reaction. Later, vasodilatation and decreased peripheral resistance lead to hypotension. The JHR is a transient clinical event and as such, no specific tests are available to diagnose the condition. However, a polymorphonuclear leucocytosis, lymphopenia and raised erythrocyte sedimentation rate have been reported (Singh, 1995).

### **II.8.3. Differential diagnosis**

The JHR is often mistaken for a drug reaction, especially to penicillin. Absence of features such as generalized urticaria/wheals and eosinophilia or specific antibodies to penicillin in the serum of patients with JHR, permit differentiation from penicillin allergy.

### **II.8.4. Complications**

Acute pulmonary hypertension has been documented in patients due to release of lipopolysaccharides from disintegrating spirochetes following treatment (Zifko, 1994). These molecules induce nitric oxide-mediated vasodilatation in the systemic vasculature, which leads to hypotension. Severe and prolonged hypotension may precipitate organ failures, including cardiac and renal. In contrast, the pulmonary vessels respond to lipopolysaccharide by producing thromboxane, causing vasoconstriction and consequent hypertension (Salzer, 1990).

Pregnancy-related complications including uterine contractions, premature labor (placental shock), and fetal distress (tachycardia and decelerations with marked decrease in fetal activity) can develop in pregnant women who have a JHR after treatment for early syphilis.

### **II.8.5. Management**

The JHR is a self-resolving condition that can be managed symptomatically. Patients must be forewarned about the possibility of a reaction, and the antibiotic treatment started initially must be fully completed. Complete bed rest, fluids and antipyretics such acetaminophen are adequate for mild cases associated with constitutional symptoms. It is important to note that the symptoms and duration of JHR can only be minimized; it is not possible to prevent the occurrence of JHR

entirely.

Timely administration of certain established medications prior to antimicrobial treatment, can offset the development of a severe JHR. In this regard, corticosteroids have been used but conclusive evidence of their benefit is lacking. Gudjonsson and Skog noted a favorable response with prednisolone, especially in curtailing the febrile response in early syphilis. Other aspects of the JHR (leukocytosis, lymphopenia) however, were unaffected (Gudjonsson, 1968). Graciansky et al. also recommended the prophylactic use of prednisone (30-50 mg) prior to antibiotic treatment. They reported a qualitative and quantitative reduction in the number of febrile reactions (in early syphilis), when corticosteroids were given in association with penicillin therapy (Graciansky, 1961).

A number of drugs have been tried in other spirochaetal infections. Fritzsche hypothesised that minocycline and hydroxychloroquine, in addition to being effective against *Borrelia burgdorferi*, possess immunomodulating and anti-inflammatory properties that can offset a possible JHR (Fritzsche, 2005). This however, has not been confirmed. Opioid analgesics (meptazinol) have also been found to attenuate the severity of JHR (Griffin, 1998; Teklu, 1983). Pre-treatment with anti-TNF antibodies, has been found to reduce the incidence and suppress symptoms of the reaction in patients with louse-borne relapsing fever (Fekade, 1996). On the other hand, pentoxifylline (TNF $\alpha$  antagonist) has not proven beneficial in suppressing JHR in humans, and its efficacy in animal models has shown conflicting results (Remick, 1996). Due to the anti-endotoxin properties of Polymyxine B, its probable utility in JHR has been suggested (Corrigan, 1979). Recently, a humanized-monoclonal antibody (HuScFv) against leptospirae was found to possess high therapeutic activity as an alternative to antibiotics, especially in patients with drug hypersensitivity (Maneewatch, 2009).

## **II.9. Context, research questions and objectives**

While being on duty in intensive care in New Caledonia, I regularly managed cases of suspected leptospirosis with severe presentation. However, most severe cases occurred among adults and paediatric cases were admitted to the regular paediatric unit with no need of intensive care. Local experienced clinicians were used to this situation, although without being able to provide clear explanation for this. In addition, available data on the subject was scarce in the published literature. To provide information about symptomatic leptospirosis in children requiring admission in the Pacific region, I decided to perform an observational retrospective study comparing children and adolescents' presentation and outcome (Study 1). To achieve my project, I was

generously given access to data (available from 2006 to 2012) of paediatric wards at Centre Hospitalier Territorial and Hopital Nord Koumac.

**Some leptospirosis** cases admitted in intensive care seemed to be complicated by challenging Jarisch-Herxheimer reactions. However, local practitioners professed this reaction was elusive in this spirochaetal disease. On the contrary, some other experienced clinicians having been working in endemic areas for more than 20 years faced several fatal cases of leptospirosis complicated by Jarisch-Herxheimer reactions. They subsequently monitored systematically all suspected cases of leptospirosis during the 12 hours following antibiotics administration. In addition, some of them developed specific local treatment protocols including corticosteroids before antibiotics administration with the hope to limit incidence and severity of the reaction. These controversial views regarding occurrence of Jarisch-Herxheimer reaction in leptospirosis gave me inspiration to explore further the question. I initiated my research by performing a systematic literature review on the question (Study 2), followed by case-control study based on data retrospectively collected both in New Caledonia and Futuna, where I had the opportunity to practice medicine (Study 3).

Finally, I faced another striking clinical situation: consecutive cases of reinfection by leptospirosis, including reinfection by the same serovar of leptospirosis despite presumed acquired genus-specific immuno-protection after first exposure. Although some clinicians and laboratory staff questioned the usefulness of serology in the case of presentation with recurrence, those cases encouraged me to describe and discuss further cases admitted with second episodes of leptospirosis. I was lucky enough to get full access to clinical and laboratory data of cases presenting several episodes of infection both in New Caledonia and Futuna to perform an observational retrospective study (Study 4).

The objective of this thesis was to describe neglected aspects of leptospirosis in New Caledonia and Futuna, including:

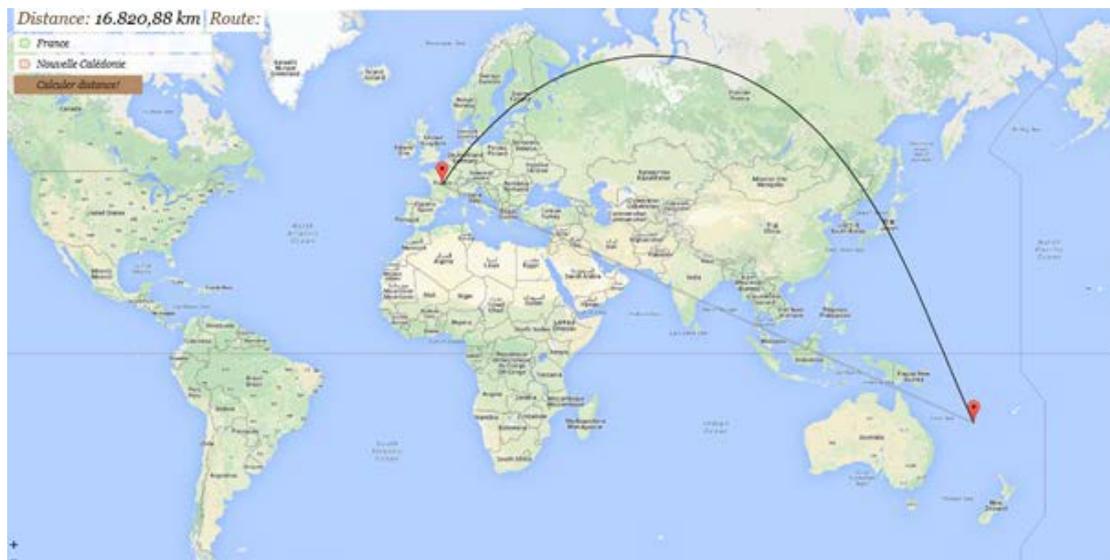
1. Epidemiology and clinical presentation of symptomatic paediatric infections
2. Prevalence and severity of Jarisch-Herxheimer reaction
3. Incidence and clinical characteristics of symptomatic reinfections

## II.10. Methodological considerations

### II.10.1. Setting

#### New Caledonia: geographic, social and economic features

New Caledonia is a special collectivity of France located in the southwest Pacific Ocean (Figure 8), divided into three Provinces (Northern, Southern and Loyalty Islands Provinces) (Figure 9). The country population was 260 000 according to the 2014 census, with 43,091 children aged 5-14 years (17.5%), among which a majority (44.6%) were indigenous Melanesian, followed by Caucasian (22.5%), Polynesian (10.9%), of mixed origins (13.0%), Asian (1.4%), and other (7.3%). The distribution of ethnic groups is uneven across the archipelago with a majority of indigenous Melanesians residing in the Northern and Loyalty Provinces. **Life expectancy is estimated at 76 years** for those born in 2010. Primary school is universal and mandatory, and attendance is estimated at 86% for primary schools (Institut national de la statistique et des études économiques, 2014a).



**Figure 8. World map.**

Although classified as a high-income country according to a GDP per capita of 35,298 USD in 2010 (rank 33) (United Nations data, 2016), with a rapidly growing market economy, major socio-economic disparities prevail within the New Caledonian society. The annual income per annum is two fold higher in the Southern Province where a majority of the population from European

descent lives compared to the other two Provinces (mainly populated by indigenous Melanesians) (Institut national de la statistique et des études économiques, 2014b).



**Figure 9. Map of New Caledonia.**

### **The New Caledonian healthcare system**

The healthcare system is similar to that of mainland France. There is universal healthcare coverage through various social security systems according to the level of income of the patient. Primary care centres are widespread and cover the entire country (Figure 10). The density of medical doctors is 223 per 100 000 inhabitants, including general practitioners and specialists. These figures vary however according to the region with 274 practitioners per 100 000 in the South, 96 per 100 000 in the North and 80 per 100 000 on the Loyalty Islands (Direction des affaires sanitaires et sociales de la Nouvelle-Calédonie, 2008).



**Figure 10. Distribution of primary healthcare centres, including primary care centres (dispensaire) and pharmacies (pharmacie).**

As in mainland France, the healthcare system in New Caledonia is dual, public and private, the latter being funded by the social security that decides the fares. The Centre Hospitalier Territorial de Nouvelle Calédonie (CHT) is the main hospital for the entire country, the only to provide specialist care including cardiology, infectious diseases, neurology, paediatrics and intensive care.

Climate in New Caledonia is marked by a cool and dry season (from June to September) and a warm and wet one (from December to March). Leptospirosis is endemic to New Caledonia, and is a leading cause of hospital admission during the rainy season. From 2006 to 2009, the average monthly incidence was 45 cases per 100,000 inhabitants but reached 150 per 100,000 inhabitants during the rainiest months. Two third of patients admitted for leptospirosis in New Caledonia are men of Melanesian background living in tribes in rural area.

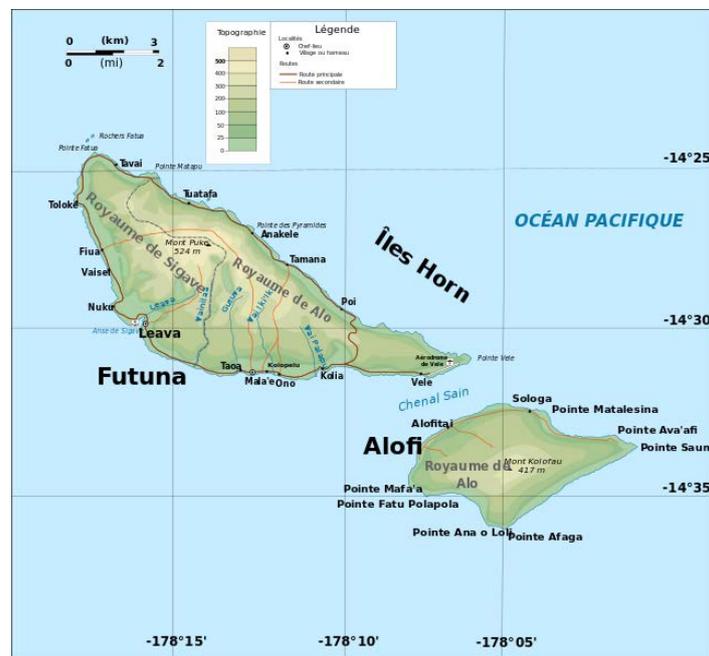
### **Futuna**

Futuna is a small (64 sq. km), mountainous (Mount Puke at 524 m) tropical island in the South Pacific, located around 2000 km North-East of New Caledonia (Figure 11). The 3,612 inhabitants (2013 census) live in 14 villages along the coast. The population is declining (4,873 inhabitants at the earlier 2003 census) mostly due to emigration of young active adults. The climate is hot and humid, with high rainfall totaling 3,000 to 4,000 millimeters a year. Futuna (together with the uninhabited island of Alofi and the island of Wallis) belongs to the French collectivity “Wallis and Futuna”. Futuna is characterized by its geographical isolation, with a single one-hour flight from Wallis weekly. The lifestyle is traditional: pig farming and irrigated taro cultivation are the main

means of subsistence and most people walk barefoot.

The economy is limited to traditional subsistence farming, with about 80% of the labour force involved in agriculture (coconuts and vegetables), livestock (mostly pigs) and fishing. About 4% of the population is employed by the government. Revenues come from subsidies from the French Government, licensing of fishing rights to Japan and the Republic of Korea, import taxes, and remittances from expatriate workers in New Caledonia. Gross domestic product (GDP) per capita was estimated at US\$ 3800 in 2004 (World Health Organization, 2011). There is one hospital and two dispensaries in Futuna. All treatment, including inpatient care, is free of charge. Futuna hospital comprises one emergency ward, one internal medicine ward with 15 beds, one post-delivery ward with seven beds, one labour ward, one laboratory, one X-ray and ultrasound unit, one pharmacy, one dental unit, and one medical evacuation unit.

The annual incidence of leptospirosis in the island was particularly high from 2005 to 2009 with an average of 1085 cases per 100,000 during this period (Massenet, 2015).



**Figure 11. Map of Futuna.**

## II.10.2. Definitions

### Leptospirosis

Leptospirosis was defined by a compatible clinical syndrome (any combination of chills, myalgia, jaundice, conjunctival suffusion, renal failure, hemorrhage, or pulmonary failure with or without fever) and laboratory confirmation with one or more of the following features: 1) positive results for a microscopic agglutination test (MAT) (using a panel of 12 serovars) (Table 9) and one acute-phase serum sample titer > 1:800, 2) seroconversion between times of testing acute-phase and convalescent phase serum samples, 3) a four-fold increase in titers between two examinations, or 4) a positive PCR. An in house-PCR targeting local pathogenic strains and elaborated by Institut Pasteur in New Caledonia was performed using serum matrix. Biological tests were performed at the Institut Pasteur in New Caledonia (IPNC).

Species	Serogroup	Serovar	Strain
<i>L. interrogans</i>	Australis	Australis	Ballico
<i>L. interrogans</i>	Autumnalis	Autumnalis	Akiyami A
<i>L. borgpetersenii</i>	Ballum	Ballum	Castellon 3
<i>L. interrogans</i>	Bataviae	Bataviae	Van Tienen
<i>L. interrogans</i>	Canicola	Canicola	Hond Utrecht
<i>L. interrogans</i>	Icterohaemorrhagiae	Icterohaemorrhagiae	Verdun
<i>L. interrogans</i>	Icterohaemorrhagiae	Copenhagenii	Winjberg
<i>L. noguchii</i>	Panama	Panama	CZ 214 K
<i>L. interrogans</i>	Pomona	Pomona	Pomona
<i>L. interrogans</i>	Pyrogenes	Pyrogenes	Salinem
<i>L. borgpetersenii</i>	Tarassovi	Tarassovi	Mitis Johnson
<i>L. biflexa</i>	Semarangae	Patoc	Patoc I

**Table 9. MAT panel used at Institut Pasteur in New Caledonia and Futuna.**

### Jarsich-Herxheimer reaction

JHR was defined as the combination of sudden onset of shivering or rigors, with rise in temperature, with or without a fall or a rise in blood pressure, increase of respiratory rate occurring within 6 hours after administration of the first dose of antibiotics and resolving within 24 hours. A 20% threshold change in haemodynamic or respiratory parameters was used. Since usual clinical presentation of leptospirosis includes non-measurable signs such as headache and myalgia, our JH definition differed from that suggested in guidelines for syphilis (Janier, 2014; Workowski, 2015). Since there is no validated definition of JHR in leptospirosis available in the literature, the definition used in this project was therefore developed on purpose. In order to detect early any deterioration, clinical monitoring of patients early after administration of antibiotics for suspected leptospirosis is common practice in New Caledonia and Futuna and data are reported in patients' notes.

### **II.10.3. Data sources, procedure and data collection**

For each study participant, a standardized form was retrospectively completed. Clinical manifestations and medical history were collected as mentioned in medical records. Demographic data and laboratory results were extracted from electronic records. Both methods (MAT and PCR product sequence polymorphism) were used to identify the serogroup or the putative serovar of the infecting strain.

### **II.10.4. Statistical methods**

Quantitative and qualitative variables were compared by using paired Student's t-tests and chi-square tests, respectively. When the frequency of events was  $< 5$  or values did not follow normal distributions, Fisher's Exact and Mann-Whitney tests were used.

The results are reported as median and interquartile range (IQR) or as numbers and percentages. Significance was defined as p-values less than 0.05. Crude and adjusted odds ratios (OR) and 95% CI were calculated. Multiple logistic regression models were constructed to identify independent factors associated with JHR. Initial regression models were first constructed including all variables for which the p-value (for the OR) was less than 0.25 in the univariate analysis. If two or more factors were highly correlated, only the most plausible one (from the literature) or the one with the fewest missing values was included in the model. To simplify the model, variables were removed one at a time depending on the significance test ( $p < 0.05$ ) by the likelihood ratio test. STATA Statistical Software: release 11 (Stat Corp., College Station, TX) was used for analysis.

### **II.10.5. Ethical considerations**

All three studies were approved by the Institutional Review Board of Centre Hospitalier Territorial. Informed consents were not obtained from the patients as this was a retrospective study. All data were anonymized. However, all patients were informed and consented that the data included in their notification files at the time of samples' collection could be used for epidemiological purposes after anonymization.

## **II.11. Studies**

### **II.11.1. Study 1: Association between age and severity to leptospirosis in children**

#### **Abstract**

#### **Background**

In endemic areas, leptospirosis is more common and more severe in adults compared with children. Reasons to explain this discrepancy remain unclear and limited data focusing on

adolescents are available. The objective of the study was to describe disease spectrum and outcome differences in children and adolescents admitted for leptospirosis in a large at-risk population.

### **Methods**

Clinical and laboratory data were obtained on hospitalized cases in New Caledonia from 2006 to 2012.

### **Results**

Data of 60 patients < 18 years of age (25 children under 14 and 35 adolescents aged 14 to 17) with confirmed leptospirosis were analyzed. Compared with children, adolescents presented more often with classic features of Weil disease ( $p=0.02$ ), combining hepatic and renal involvement with or without pulmonary participation. Jarisch-Herxheimer reactions were observed more often among adolescents ( $p<0.01$ ). The overall case fatality rate was low (1 adolescent versus 0 children).

### **Conclusion**

Severe leptospirosis in adolescents may be more likely to show adults' characteristics compared with children. Further studies are required to explore age-dependant host factors, including puberty-related physiological changes.

### **Introduction**

Along with experienced clinicians' beliefs, several studies suggest that leptospirosis produces more severe presentation in adults compared with children (91-94). Pathogen as well as host-related factors are believed to play a role in the development of severe leptospirosis in adults (44). However, factors responsible for the milder presentation among children remain unclear. There is limited information available about symptomatic leptospirosis in the under 18 age group in the Pacific region.

### **Methods**

We carried out an observational retrospective study among patients aged under 18 years old with a biologically confirmed leptospirosis admitted between January 2006 and December 2012 in either of two public hospitals (Centre Hospitalier Territorial, Noumea and Centre Hospitalier du Nord, Koumac). Demographic, epidemiologic, clinical, and laboratory information were recorded. Outpatients testing results for leptospirosis or adults over 18 were not included in the analysis.

Patients were stratified by age group: 0–13 years of age (children) and >13 years (adolescents). Oliguria was defined as urine output < 0.5 mL/kg/hour and pulmonary involvement was defined as dyspnea, rales, and/or chest radiographic abnormalities. Laboratory results refer to samples

collected at the time of admission. Reference values were serum creatinine =30–88  $\mu\text{mol/L}$ , total bilirubin = 0.8–1.2 mg/dL, and platelet counts = 150 000–400 000/mm<sup>3</sup>. The time (in days) between onset of symptoms and hospitalization was compared between groups. Occurrence of Jarisch-Herxheimer reaction (JHR) was also noted when reported. JHR was defined as the combination of sudden onset of shivering or rigors, with rise in temperature, with or without a fall or a rise in blood pressure, increase of respiratory rate occurring after administration of the first dose of antibiotics.

## Results

A total of 128 patients with leptospirosis were diagnosed during the study period. Sixty-eight of these cases were excluded from further analysis because they were not hospitalized in one of the two participating centres (n= 50) or records could not be traced (n= 16).

Of the 60 patients included in the study, 25 (42%) were children and 35 (58%) were adolescents. The majority of study subjects were boys (79% in children vs 77% in adolescents, p=0.6) with a median age of 9 [IQR 6-11] years for children and a median age of 16 [IQR 14-17] for adolescents. Subjects were mostly Melanesian (83%) living in tribes in rural areas (85%) (Table 10).

Characteristic	Children n (%)	Adolescents n (%)	p-value
Gender			
Male	18 (72)	27 (77)	0.6
Female	7 (28)	8 (23)	
Age (Mean [25–75 IQR])	9 [6–11]	16 [14–17]	
Ethnic group			
Melanesian	19 (76)	31 (88)	0.3
Other community	6 (24)	4 (12)	

**Table 10. Demographic characteristics and medical histories in 60 paediatric leptospirosis in New Caledonia, 2006-2012.**

The frequency of symptoms and complications are presented in Table 11 and biological parameters are presented in Table 12. Fever and jaundice were more frequent among adolescents whereas the

incidence of conjunctival suffusion, myalgia, abdominal pain, headache and oliguria were not significantly different between groups. Mean total serum bilirubin and serum creatinine levels were higher in adolescent than paediatric groups (41 mg/dL versus 7 mg/dL;  $p < 0.001$  and 108  $\mu\text{mol/L}$  versus 59  $\mu\text{mol/L}$ ;  $p < 0.001$ , respectively). Platelet count was lower in adolescents (148 Giga/L [95%CI 124-173]) than in children (202 Giga/L [95%CI 157-248]) ( $p = 0.02$ ). Compared with children, adolescents presented more often with classic features of Weil disease ( $p = 0.02$ ), combining hepatic and renal involvement with or without pulmonary participation. The groups showed no differences with respect to pulmonary involvement.

Clinical presentation at admission	Children n (%)	Adolescents n (%)	p-value
Fever (>38°) at admission	10 (40)	23 (66)	0.04
Jaundice	1 (4)	10 (28)	0.03
Headache	16 (64)	27 (77)	0.3
Abdominal pain	11 (44)	22 (63)	0.1
Myalgia	18 (72)	25 (71)	0.9
Conjunctival suffusion	4 (16)	9 (26)	0.4
Oliguria	2 (8)	7 (20)	0.2
Pulmonary involvement (cough, dyspnea, auscultation abnormalities)	7 (28)	11 (31)	0.6
Weil disease	0 (0)	7 (20)	0.02
<b>Complications</b>			
Alveolar hemorrhage	1 (4)	5 (14)	0.2
Myocarditis	0 (0)	1 (3)	0.5
Case-fatality rate	0 (0)	1 (3)	0.5

**Table 11. Clinical presentation in 60 paediatric (25 children and 35 adolescents) leptospirosis in New Caledonia, 2006-2012.**

Parameter*	Children n=25	Adolescents n=35	p-value
Serum total Bilirubin (mg/dL)	7 [5-12]	41 [34-55]	<0.001
Creatinine ( $\mu\text{mol/L}$ )	59 [62-65]	108 [85-132]	<0.001
Platelet count (Giga/L)	202 [157-248]	148 [124-173]	0.02

\*mean [95%CI]

**Table 12. Initial laboratory findings in 60 children admitted for leptospirosis in New Caledonia, 2006-2012.**

Exposure factors for leptospirosis transmission did not differ between the groups; most patients in both groups had self-reported direct contact with water (swimming in rivers or canals, wading through water) (87% in children and 81% in adolescents), and direct or indirect contact with potential mammalian carriers (25% in children and 28% in adolescents).

The median time between onset of symptoms and initiation of antibiotics was 2 days for both groups. Antibiotics were administered intravenously for 5-7 days and included ampicillin (100 mg/kg of body weight/day) in 43 patients (18 children and 25 adolescents) and cefotaxime (100

mg/kg of body weight/day) in 17 patients (7 children and 10 adolescents). Jarisch-Herxheimer reactions were observed more often among adolescents ( $p < 0.01$ ) (Table 13). The overall case fatality rate was 1.6% (1 adolescent versus 0 children). The single patient with fatal outcome required dialysis for acute renal failure, mechanical ventilation for alveolar hemorrhage and vasoactive drug for shock. Four other adolescents required vasoactive drugs only. No other patients required dialysis or mechanical ventilation.

	Children n (%)	Adolescents n (%)	p-value
<b>Infection serogroup</b>			
<i>L. interrogans</i> serogroup Icterohaemorrhagiae	7 (64)	8 (53)	0.7
Others	4 (36)	15 (47)	
<b>Duration of symptoms before admission to hospital (days)*</b>	2 (1-3)	2 (1-3)	0.2
<b>Jarsich-Herxheimer reaction</b>	1 (4)	12 (34)	0.005

\*mean [95% CI]

**Table 13. Characteristics of infection and effects of antibiotics among 60 leptospirosis children in New Caledonia, 2006-2012.**

Among the 26 cases for whom the serogroup was identified, 15 (58%) were Icterohaemorrhagiae (7/11 in children, 8/15 in adolescents). Other detected serogroups included Australis (n=4), Pyrogenes (n=4), Canicola (n=2), and Panama (n=1) (Table 13). For the 34 remaining cases **diagnosed by PCR only**, identification of the serogroup was not possible. At the time of admission, 8 patients had acute co-infections (5 children and 3 adolescents) with viral diseases (rotavirus n=2; viral respiratory syncytial n=1), or bacterial diseases (bacteraemia with *Serratia* n=1; urinary tract infection n=2, pyodermititis n=1, tuberculosis n =1). All patients diagnosed with coinfections were leptospirosis confirmed cases by PCR.

## Discussion

This retrospective study allowed us to identify an age-dependant association with severity of leptospirosis. Similar observations in children have been reported in other settings (Cruz, 1994). The frequency of several classic severe disease manifestations were significantly lower among small children in this study compared with adolescents. This study found that a substantial proportion of hospitalized children with leptospirosis had fewer of the classic features of Weil disease than adolescents. Although severe disease caused by leptospirosis may occur in the paediatric age group, clinical and biological presentation in adolescents overlaps with the spectrum seen in adults. Since most studies performed in children did not discriminate age groups, these findings are uneasy to compare with other series. However, some characteristics of small children

in the present study were similar to what has been previously reported in Brazil (Cruz, 1995) and Thailand (Libraty, 2007). In contrast, those results differ from a previous report of 43 children, 4-14 years of age (Marotto, 1997) showing more severe manifestations of leptospirosis than the present study, including renal failure and thrombocytopenia. Several factors may explain these differences: first, different leptospiral serogroups were identified in New Caledonia; second, time to refer was potentially higher in the Brazilian study; finally, host factors and higher organism loads may lead to more severe manifestations. Conversely, presentation of severe illness in adolescents was similar to clinical and biological profile reported in adults in Brazil (Silva, 2003) and New Caledonia (Tubiana, 2013). Unfortunately, as qualitative PCRs only were performed, bacterial load could not be quantified.

Consistent with some previous studies (Suarez Hernandez, 1999; Marotto, 1997), these results showed that overall case-fatality rates in children are lower than in adults. Recently identified factors associated with severe disease in adults in the same setting included tobacco use, leptospiral serogroup and delay between onset of symptoms and initiation of antibacterial therapy (Thai, 2006). The discrepancy between children and adolescents does not appear related to differences in seroreactivity to leptospiral serogroups, which was similar in both groups. Similarly, onset of symptoms to administration of antibiotics was similar in both groups suggesting similar time to refer for children and adolescents. Host factors may contribute to this association and could include higher organism loads with increasing age. However, since risk factors for exposure were similar in children and adolescents, it is unlikely that initial bacterial inoculum was different in both groups. Age-dependant changes in innate and adaptative immune responses to leptospiral infection are plausible explanations for differences between children and adolescents. Several hypotheses regarding leptospirosis severity are based on host genetic susceptibility factors (Lingappa, 2004; Fialho, 2009) and/or on bacterial virulence (Thaipadungpanit, 2007), although the virulence mechanisms are poorly understood and are probably multifactorial (Adler, 2011).

The male predominance reported in this study both in children and adolescents is very similar to previous studies of symptomatic leptospirosis (Marotto, 1997). Gender-specific activities may explain such differences. However, it is common in New Caledonia to see boys and girls swimming in water and assisting their parents with cultivating fields. The number of symptomatic cases admitted in paediatrics is low compared with adults over the same period of time (Tubiana, 2013). This result is surprising since clinicians have a lower threshold to admit children with

suspected or confirmed leptospirosis to the hospital. Age specific activities may explain this finding.

Another major finding of the present study showed that JHRs were more likely to occur in adolescents compared to children. Although this adverse event is scarcely described in leptospirosis (McBride, 2005), the results support the presentation and outcome overlap between adolescents and adults. The incidence of JHR in children included in this study is in line with the few studies reporting JHR in paediatric cases (Marotto, 1997). In contrast, the incidence of JHR in adolescents was elevated when compared with other studies. JHR unobserved or unreported by clinicians are potential reasons for this reduced frequency. The delay before antibacterial therapy had a major impact on outcome in adults. The need for early initiation of antimicrobial therapy to reduce disease severity remains to be proven in children. However, presumptive treatment based on clinical and epidemiological evidence appears justified while waiting for the laboratory results. Modalities of antibiotics administration to prevent JHR to occur remain to be explored.

### **Limitations**

This study suffers from several limitations. First, due to the retrospective design of the study, a significant proportion of patients with untraceable records were secondary excluded of the analysis. Second, leptospiral serogroups were identified in a limited number of cases only. Third, important clinical data were missing, including presence of puberty. Finally, indications for hospitalization may be different in younger children compared to adolescents and mild disease in adolescents may not have been seen because they were not hospitalized.

### **Conclusion**

In New Caledonia, leptospirosis is responsible for a smaller number of hospitalizations in paediatrics due to milder symptomatic forms of the disease. However, leptospirosis remains a public health threat, including in the younger age group which can present with atypical signs and symptoms. Puberty may impact on the severity reported in the older age groups. Physiologic and immunologic factors associated with improved paediatric outcomes require further investigation to provide insights into the pathogenesis of severe leptospirosis.

## **II.11.2. Study 2: The Jarisch-Herxheimer reaction in leptospirosis: a systematic review**

### **Abstract**

#### **Background**

Leptospirosis is an endemo-epidemic zoonotic disease associated with potentially fatal renal, cardiovascular or pulmonary failure. Recommended treatment includes antibiotics, which may induce a Jarisch-Herxheimer reaction (JHR). Since little information on the importance of this adverse event is available, we performed this review to quantify frequency and impact of JHR in leptospirosis management.

### **Methodology/Principal Findings**

This review systematically summarizes the literature on the JHR in leptospirosis. To approach the broader aspects of the subject, articles considering the treatment of leptospirosis, national leptospirosis guidelines and textbook and technical reports of the World Health Organisation were reviewed. Publications describing JHR in leptospirosis are very limited and consist mainly of single case reports and small case series. A single randomized control trial specifically assessed the JHR occurrence, but it has never been systematically investigated in large trials. Not all guidelines and not all literature on leptospirosis mention this reaction which can be fatal.

### **Conclusions/Significance**

Although generally assumed to be a rare event, the true prevalence of JHR in leptospirosis is unknown and the awareness of this event is insufficient. All leptospirosis guidelines and local leptospirosis protocols should **stress systematic** monitoring for clinical status early after antibiotic administration. Large well designed studies are **required to more precisely estimate the incidence** and the impact of JHR as well as the severity and rates between various antibiotics.

### **Introduction**

Leptospirosis has a wide-ranging clinical spectrum from asymptomatic forms to severe presentations. The latter are estimated to occur in 5-15% of all human infections and commonly combine jaundice, renal failure, myocarditis and/or haemorrhage (McBride, 2005) with a significant mortality rate. The benefit of antibiotic therapy on mortality **lacks clinical** evidence (Taylor, 2015). However, the current treatment guidelines still rely on antibiotic administration regardless of stage or severity of the disease (World Health Organization, 2003). Initiation of chemotherapy in spirochetal diseases may precipitate a febrile inflammatory reaction (Bryceson, 1976), known as the Jarisch-Herxheimer reaction, originally described in patients with syphilis receiving mercury treatment (Herxheimer, 1902). This reaction is characterised by an acute inflammatory response associated with the release of large amounts of cytokines, **associated** with clearance of spirochetes from the circulation. Prevalence, clinical manifestations and outcome of JHR have been well studied in syphilis (Singh, 1995), Lyme disease (Maloy, 1998), tick-born relapsing fever (Dworkin, 1998) and louse-born relapsing fever (Warrell, 1983). However, a clear

idea of importance JHR in leptospirosis is lacking. In order to quantify frequency and impact of JHR in leptospirosis, we systematically reviewed the published literature and put it in a broader perspective, identifying data gaps that should be addressed.

## **Methods**

### *Search strategy and selection criteria*

The inclusion criteria were as follows: all publications on leptospirosis treated with antibiotics providing information about the occurrence of early adverse events including JHR were selected. When the original article was not obtainable but the abstract containing the requested information was, the publication was included in the analysis. To avoid multiple counting (duplication) of identical procedures and cases, follow-up publications on identical procedures and cases were traced and excluded. The electronic database PubMed was searched with the keywords “leptospirosis” and “Leptospira”. A second search was performed for “penicillin”, “amoxicillin”, “ampicillin”, “cefepime”, “ceftriaxone” and “antibiotics”. A final search was done with “Jarisch-Herxheimer reaction”. Search results were assessed using different combinations. **The search was not limited by study design, language or date of publication until November 30<sup>th</sup> 2012.** A similar search was conducted with Embase. The Cochrane Library was also searched for leptospirosis publications. World Health Organization (WHO) websites and international or accessible national guidelines in endemic countries were searched, and the latest editions of five standard textbooks in infectious and tropical diseases were studied (Cook, 2008; Gorbach, 2004; Guerrant, 2011; Mandell, 2009; Warrell, 2012). Titles and abstracts were screened, and potentially relevant case reports, case series and trials were further evaluated. Reports were included if they assessed the occurrence of JHR in leptospirosis. Reference lists of included articles were screened for further relevant publications. Study identification and data extraction were conducted independently and cross-checked for accuracy by both authors. **PRISMA guidelines were followed and Prospero statement was completed.**

### *Data abstraction and methodological assessment*

The primary outcome was JHR occurrence or rates measured at time points described by investigators. Secondary outcomes include renal failure rates and mortality. Serovars were also recorded. It was impossible to retrospectively grade the severity of the JHR due to the lack of a standardized definition of the events.

## **Results**

Twenty-eight publications met the inclusion criteria, with a total number of 976 leptospirosis cases treated with antibiotics. Abstract or full text was not found for an additional publication which was also excluded (Munnich, 1972). One previous review dedicated to the topic was published in 1991 without systematically assessing the literature (Friedland, 1991). Among the selected studies, 11 patients presenting with concomitant infections were excluded (Phimka, 2007).

#### *Studies characteristics and JHR occurrence*

Eight case reports ( Markham, 2012; Hashimoto, 2012; Masuda, 2010; Tattevin, 2003; Emmanouilides, 1994; Swiader, 1995; Lau, 2012; Crooks, 1955) and five case series ( Phimda, 2007; Narita, 2005; Vaughan, 1994; Winearls, 1984; Mackay-Dick,1957) published between 1955 and 2012 were reviewed and analyzed. All selected patients had a laboratory confirmed leptospirosis without concomitant infection and all were treated with antibiotics. A JHR was reported in 92 patients from 1 to 48 hours after administration of the first dose of antibiotics. The nature of antibiotics regimen given varied considerably: penicillin (n=81), ampicillin (n=8), ceftriaxone (n=1), cefepime (n=1) and penicillin and ceftriaxone (n=1). The most common features of described JHR were sudden onset of shivering or rigors (n=6), with rise in temperature (n=9), with (n=8) or without (n=1) a fall in blood pressure, occurring after administration of the first dose of antibiotics (Table 14). The largest case series accounted for 70 cases, out of 84 case-patients treated with penicillin in Malaya in 1957 ( Mackay-Dick, 1957).

Seven randomized control trials (RCT) (Costa, 2003; Edwards, 1988; McClain, 1984; Panaphut, 2003; Phimda, 2007; Suputtamongkol , 2004; Watt, 1988; Watt, 1990), six non-randomized control trials (NRCT) (Doherty, 1955; Fairburn, 1956; Hall, 1951; McClain, 1984; Russell, 1958; Watt G 1990) were reviewed and analyzed (Table 15). With the exception of two RCTs (Costa, 2003; Edwards, 1988), all studies monitoring the efficacy of antibiotics were not designed to assess adverse events linked to antibiotics including the JHR occurrence. The single study designed to monitor adverse events focusing on leptospirosis-related JHR was performed in 1986 by Watt et al. (Watt, 1988). In this study, a single Herxheimer-like reaction was observed in a patient receiving saline placebo. The authors concluded that fear of a Herxheimer-like reaction should not dissuade clinicians from administering antibiotics to patients with leptospirosis. However, studied patients received up to four doses of parenteral antibiotic or had completed up to two days of an oral antibiotic regimen before inclusion.

#### *JHR in national and international guidelines, textbooks and WHO/ILS guideline*

Among 3 screened national or international guidelines, all described JHR as a potential adverse event, although occurring with penicillin only (World Health Organization, 2003; Disease control division Doph, Ministry of Health, Malaysia, 2011; Philippine College of Physicians, 2011). Three textbooks out of the five consulted mentioned the possibility of JHR early after penicillin initiation (Cook, 2008; Warrell, 2012; Guerrant, 2011).

#### *JHR management and outcome*

JHR management was not specified in 82 cases, but reported with no details in five cases (supportive care). In the remaining cases, the management consisted in fluid infusion, vasopressors (n=1), corticosteroids and inotropic support (n=1) and transient dialysis (n=1). Overall, eleven studies mentioned immediate outcome after JHR occurrence. Among them, all cases fully recovered with the exception of one JHR-related death (Narita, 2005).

#### *JHR and Leptospira serovars*

Out of 13 case reports or case series reporting JHR, the pathogen serovar was stated in 18 patients. Nine different types of serovar were involved in JHR-patients, the most prevalent being Icterohaemorrhagiae (n=6).

References	Country	Study design	Treatment administered	Delay before JHR	Symptoms	Outcome
Lau CL et al. Emerg Infect Dis 2012	American Samoa	Case report (n = 1)	penicillin	1 hour	Increase in fever Rigors Severe Headache	Discharged
Markham R et al. Med J Aust 2012	Australia	Case report (n = 1)	benzylpenicillin and ceftriaxone	2 hours	Tachycardia Tachypnea Hypertension Severe rigors	Discharged
Hashimoto T et al. J Thorac Imaging 2012	Japan	Case report (n = 1)	ceftriaxone	2 days	Pulmonary deterioration	Discharged
Masuda K et al. Kansenshogaku Zasshi 2010	Japan	Case report (n = 1)	cefepime	Unknown	Chills Fever Hypotension	Discharged
Narita M et al. Am J Trop Med Hyg 2005	Japan	Case series (n = 6)	ampicillin (n = 6)	Unknown	Rigors Hypotension	Unknown
Tattevin P et al. Eur J Clin Microbiol Infect Dis 2003	France	Case report (n = 1)	amoxicillin	4 hours	Headache Hypotension Tachycardia Fever Nuchal rigidity	Discharged
Swiader L et al. La Presse Med 1995	France	Case report (n = 1)	penicillin	8 hours	Headache Photophobia Nuchal rigidity	Unknown
Vaughan C et al. Postgrad Med J 1994	Ireland	Case series (n = 3)	benzylpenicillin (n = 2) ampicillin (n = 1)	4 to 5 hours	Increase in fever Hypotension	Died (n = 1) Discharged (n = 2)
Emmanouilides CE et al. Clin Infect Dis 1991	USA	Case report (n = 1)	penicillin	Few hours	Fever Chills Hypotension Respiratory distress	Discharged
Friedland JS et al. Rev Infect Dis 1991	UK	Case series (n = 2)	benzylpenicillin (n = 2)	5 hours 4 hours	Fever Severe rigor Hypotension Abdominal pain Headache Fever Profuse vomiting	Discharged
Winearls CG et al. Q J Med 1984	UK	Cases series (n = 3)	penicillin (n = 3)	Unknown	Sharp rise in temperature Rigor	Discharged
Mackay-Dick J et al. J Royal Army Medical Corps 1957	Malaysia	Case series (n = 70)	penicillin (n = 70)	Unknown	Fever (n = 59) Aggravation of classical symptoms (n = 31) Hypotension (n = 58) Oligo-anuric (all)	Discharged
Crooks J et al. Br Med J	Scotland	Case report (n = 1)	penicillin	2 ½ hours	Rigor Fever	Discharged

**Table 14. Case reports and case series of Jarsich-Herxheimer reaction after administration of antibiotics for the treatment of leptospirosis.**

References	Country Year of study	Study design	Treatment	Any adverse events reported	Systematic assessment of JHR	Observation of JHR	Delay before JHR	Symptoms of JHR	Outcome
Phimda K et al. Antimicrob Agents Chemother 2007	Thailand 2003–2005	RCT	doxycycline (n = 34) vs azythromycin (n = 35)	Rash Nausea Vomiting	Unknown	No	NA	NA	No death
Suputtamongkol Y et al. Clin Infect Dis 2004	Thailand 2001–2002	RCT	penicillin (n = 52) vs cefotaxime (n = 59)	Diarrhea Abdominal pain Dizziness Skin rash	Unknown	No	NA	NA	4 deaths
Panaphut T et al. Clin Infect Dis 2003	Thailand 2000–2001	RCT	doxycycline (n = 53) vs ceftriaxone (n = 87)	No	Unknown	No	NA	NA	10 deaths
Costa E et al. Rev Inst Med Trop Sao Paulo 2003	Brazil 1997–1999	RCT	penicillin (n = 86) vs penicillin (n = 125)	No	Unknown	No	NA	NA	15 deaths
Daiher EdF et al. Rev Inst Med Trop Sao Paulo 2000	Brazil 1996–1998	NRCT	placebo (n = 128) vs penicillin (n = 16)	No	Yes	No	NA	NA	1 death
Marotto PCF et al. Ann J Trop Med Hyg 1997	Brazil 1989–1995	NRCT (retrospective analysis of children)	untreated (n = 19) vs penicillin or ampicillin (n = 28)	No	Unknown	No	NA	NA	1 death
Watt G et al. J Infect Dis 1990	Philippines 1985–1986	RCT	penicillin (n = 24) vs placebo (n = 16)	No	Yes	Yes (n = 1 in patient receiving placebo)	Unknown	Unknown	No death
Edwards CN et al. Ann J Trop Med Hyg 1986	Barbados 1983–1986	RCT	penicillin (n = 38) vs placebo (n = 41)	No	Unknown	Yes (n = 1 in patient receiving penicillin)	3 hours	Unknown	4 deaths
McClain BL et al. Ann Intern Med 1984	Panama Year not specified	RCT	doxycycline (n = 14) vs placebo (n = 15)	No	Yes	No	NA	NA	No death

References	Country Year of study	Study design	Treatment	Any adverse events reported	Systematic assessment of JHR	Observation of JHR	Delay before JHR	Symptoms of JHR	Outcome
Russel RW et al. Lancet 1958	Malaya Year not specified	NRCT	oxytetracycline (n = 27) vs placebo (n = 25)	Pyrexia Sore throat Erythema Urticaria	No	No	NA	NA	Unknown
Doherty RL et al. Australas annals of Medicine 1955	Australia Year not specified	NRCT	penicillin (n = 71) vs chlormphenicol (n = 12)	No	No	No	NA	NA	Unknown
Fairburn AC et al. Lancet 1956	Malaya Year not specified	NRCT	chlormphenicol+penicillin (n = 20) penicillin (n = 10) vs chlormphenicol (n = 14)	No	No	No	NA	NA	No death
Hall HE et al. Annals of Internal Medicine 1951	Puerto Rico Year not specified	NRCT	placebo (n = 22) penicillin (n = 5) vs chlormphenicol (n = 18)	No	No	No	NA	NA	2 deaths
			aureomycin (n = 13) vs terramycin (n = 8)						
			streptomycin (n = 12) vs aureomycin+streptomycin (n = 9)						
			aureomycin+cortison (n = 2) vs untreated (n = 12)						

RCT: Randomized Control Trial; NRCT: Non Randomized Control Trial.

**Table 15. Clinical trials for leptospirosis treatment in which adverse events were searched.**

## Discussion

To the best of our knowledge, this systematic review is the first to formally appraise the measurement of JHR occurrence in leptospirosis treated with antibiotics. Publications describing JHR consist mainly of single case reports or case series. All RCTs included in this review but one were not designed to assess JHR incidence and therefore judged to be of poor methodological quality. Moreover, methods used to monitor JHR were not described in enough detail to reach predefined criteria and to determine whether there was a link between JHR occurrence and organ failure or death. Most reports were targeted at criteria such as duration of fever, clearance of spirochetes and length of stay. The single appropriately designed and detailed RCT had major limitations, including a small number of subjects and a lack of JHR confirmation.

However, this work will help to better estimate the potentially detrimental impact of antibiotics in leptospirosis and inform future management guidelines. Since there is little doubt that renal damage can be aggravated by vascular hypotension, particular attention should be paid to monitor blood pressure after initiation of antibiotics in suspected leptospirosis, especially in particular conditions such as pregnant, hypotensive or chronic renal insufficiency presenting patients.

It is postulated that the inflammatory process results from activation of the cytokine cascade during the degeneration of spirochetes. Therefore, the apparent lower proportion of JHR in patients with leptospirosis compared with patients diagnosed with other spirochetal diseases may be explained by a lower bacteraemia. This hypothesis requires further investigation for validation. JHR unobserved or unreported by clinicians is an additional reason for this ostensible reduced frequency. Conversely, a confusion of JHR symptoms with the aggravation of the leptospirosis itself may lead to overestimate the incidence of this event.

Interestingly, international guidelines for the management of leptospirosis briefly mention the occurrence of JHR with penicillin, omitting the details of its prevention, management or outcome. Moreover, antibiotics other than penicillin (e.g. ceftriaxone) are not evoked. Similarly, infectious diseases textbooks mentioning the event overlook this reaction with non-penicillin antibiotics. Noteworthy, recommended antibiotics for the management of leptospirosis vary considerably according to settings. For example, most commonly used drugs in the Philippines for mild leptospirosis is **doxycycline** while penicillin G is recommended for treating severe cases (Philippine College of Physicians, 2011); amoxicillin and **cefotaxime** are preferentially used in French Caribbean or in New Caledonia in mild and severe cases, respectively.

Some limitations in the current review should be acknowledged. First, potential publication bias is impossible to completely exclude. Moreover, unpublished reports or reports that were not referenced in databases could have been missed despite the comprehensive search. Second, patients presenting with undetected co-infections may have been included in the study. Finally and most importantly, the diagnosis of JHR was not supported by any **data on concentration** of biological markers. In addition, the definition of JHR was not uniform, including in the largest case series published by Mackay-Dick (Mackay-Dick, 1957) which lacks of details such as the onset of reaction. Therefore, although most of the cases presented a genuine JHR, some might have had a clinical aggravation related to the spirochetal disease regardless of the antibiotherapy.

### **Conclusion**

The prevalence of JHR in leptospirosis treated with antibiotics is unknown and the awareness of this adverse event is insufficient. Although this review suggests that antibiotic treatment in patients with leptospirosis may result in less common and less severe JHR than in patients with other spirochetal diseases, all leptospirosis guidelines and local leptospirosis protocols should stress on systematic monitoring for clinical status early after antibiotic administration. We strongly recommend that patients receiving penicillin or other antibiotics for the management of leptospirosis to be monitored early after initiation of treatment to prevent any detrimental effects of a potential JHR. Since it is still controversial whether antimicrobials produce a beneficial effect in mild human leptospirosis, large well designed studies are required integrating a specific monitoring to precise the incidence and the impact of JHR as well as the severity and rates between various antibiotics.

### **II.11.3. Study 3: Jarisch-Herxheimer reaction among patients with leptospirosis: incidence and risk factors**

#### **Abstract**

#### **Background**

A Jarisch-Herxheimer reaction (JHR) may be precipitated after initiation of chemotherapy in spirochaetal diseases, including leptospirosis. However, a clear idea of the importance of JHR in this disease is lacking.

#### **Methods**

The incidence of and risk factors for JHR were investigated retrospectively among 262 patients with confirmed leptospirosis who received amoxicillin treatment in New Caledonia and Futuna.

#### **Results**

The overall rate of JHR was 21% (12% in New Caledonia and 44% in Futuna). Two risk factors were independently associated with JHR occurrence: *Leptospira interrogans* serogroup Australis as the infecting strain (OR=2.60 [CI 1.40-5.62]) and delays <3 days between the onset of symptoms and the initiation of antibiotherapy (OR=2.14 [CI 1.11-4.38]).

#### **Conclusions**

Clinicians should be aware of JHR as a potential complication of leptospirosis. Strain-related factors associated with JHR occurrence and its impact on outcome remain to be explored.

#### **Introduction**

In accordance with current treatment guidelines, the management of leptospirosis relies on antibiotic administration regardless of stage or severity of the disease (World Health Organization, 2003). Initiation of chemotherapy in spirochaetal diseases may precipitate a febrile inflammatory reaction, known as the Jarisch-Herxheimer reaction (JHR), originally described in patients with syphilis receiving mercury treatment (Jarisch, 1895; Herxheimer, 1902). This reaction is characterized by an acute inflammatory response with release of large amounts of cytokines as a result of exposure to antigen released from lysis during a high quantity spirochaetemic phase of infection (Bryceson, 1976; Pound, 2005). Contrary to syphilis (Janier, 2014), Lyme disease (Maloy, 1998), tick-born relapsing fever (Dworkin, 1998) and louse-born relapsing fever (Bryceson, 1972), the overall rate of JHR for leptospirosis is not well documented (Guerrier, 2013). In addition, factors associated with this complication are not clearly established. In this study, we aimed to investigate the incidence of and risk factors for JHR among patients admitted for leptospirosis in two Pacific islands after receipt of amoxicillin.

## Methods

We carried out an observational retrospective study among patients with a biologically confirmed leptospirosis admitted between January 2007 and December 2009 in either of two public hospitals (Centre Hospitalier Territorial, Noumea and Centre Hospitalier du Nord, Koumac) in New Caledonia or in the single medical centre in Futuna. Outpatients testing results for leptospirosis or patients with antibiotic treatment received prior to admission without clinical monitoring were not included in the analysis. **Leptospirosis was defined by a compatible clinical syndrome (any combination of chills, myalgia, jaundice, conjunctival suffusion, renal failure, hemorrhage, or pulmonary failure with or without fever)** and laboratory confirmation with one or more of the following features: 1) positive results for a microscopic agglutination test (using a panel of 11 serovars) **(Table 9)** and one acute-phase serum sample titer >1:800, 2) seroconversion between times of testing acute-phase and convalescent phase serum samples, 3) a four-fold increase in titers between two examinations, or 4) a positive PCR.

JHR was defined as the combination of sudden onset of shivering or rigors, with rise in temperature, with or without a fall or a rise in blood pressure, increase of respiratory rate occurring within 6 hours after administration of the first dose of antibiotics and resolving within 24 hours. A 20% threshold change in haemodynamic or respiratory parameters was used. Since usual clinical presentation of leptospirosis includes non-measurable signs such as headache and myalgia, the JH definition differed from that suggested in guidelines for syphilis (Janier, 2014; Workowski, 2015). In order to detect early any deterioration, clinical monitoring of patients early after administration of antibiotics for suspected leptospirosis is common practice in New Caledonia and Futuna and data are reported in patients' notes. In practice, tympanic temperature, blood pressure, and respiratory rate measurements were taken every two hours for six hours and then every 6 hours for 24 hours.

## Results

During the study period, 262 patients with confirmed leptospirosis who received antibiotic treatment were included in New Caledonia (n=191) and Futuna (n=71). The demographic characteristics of patients with leptospirosis and the clinical presentation of JHR were similar in New Caledonia and Futuna (Table 16). The overall incidence of JHR was 21% (95% CI, 18%–30%) with a higher incidence in Futuna (44%) compared with New Caledonia (12%) (p<0.001). The spectrum of JHR is shown in Table 16. The median time to development of JHR was 4 hours

(interquartile range, 2–5 hours) for the 2 groups. Fever resolved within 1 to 4 hours (spontaneously or with antipyretics).

Characteristics	Total n=262	New Caledonia n=191	Futuna n=71	P
Age, mean ± SD (years)	35 ± 10.7	36 ± 12.1	34 ± 9.1	0.9
Male sex	206 (79)	150 (79)	56 (71)	0.95
Jarisch-Herxheimer reaction	54 (21)	23 (12)	31 (44)	<0.001
<b>Type of reaction</b>				
Shivering/rigors with rise in temperature	54 (100)	23 (100)	31 (100)	0.95
Fall in blood pressure	26 (48)	11 (49)	15 (47)	0.85
Rise in blood pressure	20 (37)	8 (33)	12 (40)	0.08
Increase of respiratory rate	45 (83)	20 (89)	25 (78)	0.09
Onset of JH reaction following amoxicillin treatment, hours (IQR)	4 (2-5)	4 (2-5)	4 (2-5)	0.95
<b>Infecting serogroup (n=217)</b>				
Icterohaemorrhagiae	94/217	89 (95)	5 (5)	<0.001
Australis	91/217	32 (35)	59 (65)	0.01
Other	32/217	20 (62)	12 (38)	<0.001
<b>Laboratory identification method</b>				
PCR	164/217	132 (61)	32 (19)	
MAT	53/217	33 (62)	20 (38)	
Transfer to intensive care	18 (100)	18 (9)	0	

**Table 16. Clinical characteristics of patients admitted with confirmed leptospirosis, 2007-2009.**

Both MAT and PCR were used to identify the infecting serovar in 217 patients (83%). The most common sequence cluster type identified was linked to serogroup Icterohaemorrhagiae in 71 patients, Australis in 70, Pyrogenes in 18 patients, Bataviae in 3, Ballum in 2 patients, and Pomona in 2. Using MAT results, the serogroup involved was identified in 53 patients. Among these, 45 were confirmed cases with an acute and a convalescent serum and 8 had a single MAT titer  $\geq 1600$ . The most common serogroups were Icterohaemorrhagiae observed in 23 patients, Australis in 21, Pyrogenes in 3 patients, Panama in 1, Pomona in 1, Tarassovi in 1, Canicola in 1. For the 45 remaining cases, identification of the serogroup was not possible.

Variable		JH reaction n=54	No JH reaction n=208	OR (95% CI)	p
<b>Gender</b>	<b>Female</b>	19 (37)	79 (38)	1	0.84
	<b>Male</b>	35 (63)	129 (62)	0.94 (0.50-1.75)	
<b>Age (years)</b>	<b>Mean (SD)</b>	30 (8.5)	16 (14.5)		0.29
	<b>≤30</b>	33 (61)	131 (63)	1	
	<b>&gt;30</b>	21 (39)	77 (37)	1.37 (0.68-2.77)	
<b>Infecting serogroup</b>	<b>Other</b>	1/40 (2)	11/177 (7)	1	0.003
	<b>Icterohaemorrhagiae</b>	6/40 (15)	98/177 (55)	0.35 (0.10-0.59)	
	<b>Australis</b>	33/40 (83)	68/177 (38)	2.75 (1.30-5.82)	
<b>Delay between onset of symptoms and initiation of antibacterial therapy (days)</b>	<b>&gt;2</b>	18 (33)	97 (47)	1	0.01
	<b>0-2</b>	36 (67)	110 (53)	2.24 (1.21-4.17)	
<b>Creatinine (mM/L)</b>	<b>≤200</b>	40 (74)	170 (82)	1	0.17
	<b>&gt;200</b>	14 (26)	38 (18)	1.67 (0.80-3.50)	
<b>Platelet count (Giga/L)</b>	<b>≤50</b>	13 (36)	71 (34)	1	0.29
	<b>&gt;50</b>	41 (74)	137 (66)	1.44 (0.73-2.82)	
<b>Transfer to intensive care</b>		1 (2)	17 (8)	0.89 (0.69-1.02)	0.07

**Table 17. Univariate analysis of factors associated with Jarisch-Herxheimer reaction among patients with confirmed leptospirosis, New Caledonia and Futuna, 2007-2009.**

Comparison of clinical and biological characteristics of patients in New Caledonia and Futuna are shown in Table 17. None of the patients treated in Futuna required intensive care. There was no significant statistical difference ( $p=0.07$ ) among 18 patients transferred to intensive care unit in New Caledonia, with or without JHR (OR=0.89 (CI 0.69-1.02)). Renal failure and thrombocytopenia were not associated with JHR occurrence. Patients who developed JHR following the first dose of amoxicillin did not have recurrent symptoms after the second dose during the same course of treatment. The pregnancy status of women having JHR was not known, except for one who was obviously pregnant. The outcome was favorable both for the mother and the fetus. Not admitted women of childbearing age did not have pregnancy tests.

Two risk factors were independently associated with JHR occurrence: *Leptospira interrogans* serogroup Australis as the infecting strain (OR=2.60 [CI 1.40-5.62]) and delays <3 days between the onset of symptoms and the initiation of antibiotherapy (OR=2.14 [CI 1.11-4.38]) (Table 18).

Variable		JH reaction n=54	No JH reaction n=208	OR (95% CI)	p
Infecting serogroup	Other	7/40 (17)	109/177 (62)	1	0.009
	Australis	33/40 (83)	68/177 (38)	2.60 (1.40-5.62)	
Delay between onset of symptoms and initiation of antibacterial therapy (days)	>2	18 (33)	97 (47)	1	0.01
	0-2	36 (67)	110 (53)	2.14 (1.11-4.38)	

**Table 18. Multivariable analysis of independent factors associated with Jarisch-Herxheimer reaction among patients with confirmed leptospirosis, New Caledonia and Futuna, 2007-2009.**

### Discussion

This retrospective case-control study of leptospirosis allowed us to identify risk factors associated with JHR in two Pacific Islands. One major finding of this study was the independent association between the serogroup Australis and occurrence of JHR. Although various strains were identified in JHR in previous reports, none were clearly associated with this complication. Hypotheses regarding JHR occurrence were based more on host genetic susceptibility factors rather than bacterial characteristics. The more prevalent strain in Futuna during the study period was Australis serogroup, tied to pig farming and causing few severe cases (Massenet, 2015). This finding requires further research to better characterize strain-dependant factors potentially triggering JHR.

Multivariable analysis identified another risk factor linked to the initial management of the disease: if antibiotics were started earlier than two days after symptom onset, the risk of developing a JHR was higher than if antibiotics were started later on. Early antimicrobial therapy initiation in suggestive epidemiological and clinical contexts is recommended, even before laboratory confirmation of leptospirosis. The present association with delay which has also been found for syphilis (Janier, 2014) underscores the need for monitoring patients when initiation of antimicrobial therapy occurs early in the course of the disease. Leptospiremia was not available for most patients and therefore potential association between bacterial load and JHR occurrence could not have been analyzed. Although time from onset of symptoms to treatment was similar in both islands, a higher leptospiremia in cases treated in Futuna has possibly contributed to the observed difference in JHR incidence. **As qualitative PCRs only were performed in our study, bacterial load could not be quantified.** Since a higher load of spirochetes in the early stages of syphilis increases the risk of JHR when patients receive bactericidal agents (Janier, 2014), this aspect needs to be explored in leptospirosis.

The proportion of JHR was high in this study, suggesting that suspected cases of leptospirosis should be systematically monitored when receiving the first dose of bactericidal agents. JHR is not classically reported in studies describing prognostic factors of leptospirosis but clinicians are usually facing complicated management (Shenep, 1986). Moreover, although the JHR is usually self-limited, the reaction might be severe enough to result in cardiovascular failure with potential aggravation of renal damage. Interestingly, all cases in Futuna were treated successfully where neither intensive care nor dialysis is available, while 10% of patients admitted in New Caledonia required secondary transfer to intensive care unit, most of them without JHR. Laboratory findings known for their strong correlation with severe outcomes, including renal failure and thrombocytopenia were not associated with JHR occurrence in this study. The association between JHR and renal injury should be formally assessed in a prospective study.

Since laboratory methods of confirmation provide delayed results or are even not widely available in resource-limited settings where leptospirosis is prevalent, reliance is placed on clinical features for provisional diagnosis. However, the clinical presentation of the disease is known to mimic the clinical profile of other prevalent tropical fevers. This finding suggests that JHR occurrence in patients presenting with suspected leptospirosis in Australis serogroup prevalent area may serve as an early sign predicting leptospirosis. This clinical observation may usefully complete a model recently elaborated for diagnosis of leptospirosis, based on clinical features and standard laboratory test results (Rajapakse, 2016).

The present study suffers from several major limitations. First, the retrospective design exposed to misclassification bias, including patients with early recovery, or compensated severe sepsis. A prospective data collection using this definition would be more rigorous. Second, the definition for JHR was based on clinical signs and not supported by any dosage of biological markers. Therefore, although most of the cases presented a genuine JHR, some might have had a clinical aggravation related to the spirochaetal disease regardless of the antibiotherapy. In addition, we cannot exclude that chills and increase fever were not part of leptospirosis disease after the weaning off of the effect of antipyrexial drugs. Third, the sample size of this study remains small, which limits subgroup analysis in attempts to identify other factors for JHR, such as ethnicity. Fourth, deciding the infecting serogroup based on MAT is having a very low sensitivity and difficult to use as a predictor. Lastly, while multivariable analysis supports an association of serovar with JHR, potential host, pathogen, environmental and clinical confounding features may exist between the

two sites.

In conclusion, JHR in leptospirosis is not rare in the Pacific and deserves full attention from clinicians treating suspected leptospirosis cases. *Leptospira interrogans* serogroup Australis and short delays between the onset of symptoms and the initiation of antibiotherapy are associated with an increased risk for JHR. Strain-related factors associated with JHR occurrence and severity and its impact on outcome remain to be explored.

#### **II.11.4. Study 4: Reinfection in leptospirosis: a 3-year retrospective study in two Pacific islands**

##### **Abstract**

##### **Background**

Recurrent episodes of leptospirosis remain scarcely described. The objective of the study was to assess incidence and characteristics of reinfection by leptospirosis in a large at-risk population of the Pacific region.

##### **Methods**

We performed a longitudinal study to identify patients admitted for leptospirosis more than once over a 3-year period in New Caledonia and Futuna Island (2007-2009).

##### **Results**

Among 446 individuals admitted for confirmed leptospirosis, 5 cases of reinfection were identified in New Caledonia (2 cases) and Futuna (3 cases) during the study period. All recurrent episodes occurred in adults who presented with fever (100%), jaundice (80%), conjunctival suffusion (80%), headache (80%), and myalgia (80%). Renal function impairment was noted in 2 cases. No Weil disease nor fatality cases were observed. The mean time elapsed between the two episodes was 23 months. The serogroup was identified in all recurrent cases and was similar to the previous episode in 2 (40%) cases.

##### **Conclusions**

Recurrent episodes of symptomatic leptospirosis appear to be non-severe, possibly due to awareness of the medical community. The observed low incidence of symptomatic reinfection may be explained by the potential role played by individual immunological factors in the physiopathology of the disease.

##### **Introduction**

Leptospirosis is the most widespread zoonotic disease, infecting both human and animals. Highest morbidity and mortality are estimated to occur in resource-poor countries, which include regions where the burden of leptospirosis has been underappreciated (Costa, 2015). Infection by pathogenic strains of *Leptospira* commonly occurs through direct contact with infected animal urine or indirectly through contaminated water (Bharti, 2003). Almost every mammal can serve as a carrier of leptospire, harboring the spirochete in the proximal renal tubules of the kidneys, leading to urinary shedding. Rats serve as the major carriers in most human leptospirosis, excreting high concentrations of leptospire months after their initial infections. Humans, on the other hand, are considered as incidental hosts, suffering from acute but sometimes fatal infections.

*Leptospira* enters the body via cuts or abrasions in the skin or through mucous membranes of the eyes, nose or throat. The onset of the disease in humans is variable, ranging from 1 day to 4 weeks after exposure. Leptospirosis may present a broad variety of clinical manifestations. Several factors have been suggested to impact the severity of illness including the *Leptospira* strain or serovar involved, inoculum size for at least some strains, as well as the age, health and immune status of the infected individual. Many of the documented cases are mild and self-limited, and may be difficult to distinguish from other infectious diseases, until a laboratory diagnosis of leptospirosis is made. The severe presentations are estimated to occur in 5-15% of all human infections and commonly combine jaundice, renal failure, myocarditis and/or haemorrhage with a significant mortality rate (Spichler, 2008).

Humans react to an infection by leptospire by producing specific anti-*Leptospira* antibodies. Seroconversion may occur as early as 3–7 days after the onset of disease but sometimes only after 10 days or longer. Immunity to leptospirosis is primarily humoral. Cell-mediated immunity does not appear to be important, but may be responsible for some of the late manifestation of the disease. IgM class antibodies usually appear somewhat earlier than IgG class antibodies, and generally remain detectable for months or even years but at low titer. Detection of IgG antibodies is more variable. They may sometimes not be detected at all, or be detectable for only relatively short periods of time, but may sometimes persist for several years (Ko, 2009).

Although reinfection is well-recognized in other spirochetal diseases, such as Lyme disease (Krause, 2006) and syphilis (Magnuson, 1956), there is little detailed information on the incidence, clinical and laboratory manifestations of recurrent episodes of leptospirosis. It is generally believed that serovar-specific antibodies are protective and that a patient is immune to reinfection with the

same serovar as long as the titre of specific antibodies is high enough (Ko, 2009). Antibodies provoked by an infection with a particular serovar do not necessarily protect against infection with other serovars. The disease progression is influenced, in part, by the production of circulating antibodies directed against serovar specific LPS, by the dose of infecting inoculum, and by the virulence characteristics of the infecting strain (Srikram, 2011). A recent study performed in Azores suggested some degree of long-term protection against leptospires with an attenuation of symptoms in case of reinfection (Esteves, 2014). In order to explore this potential protection in a different setting, we performed a retrospective study on *Leptospira* reinfection incidence in two endemic areas of the Pacific region.

## **Methods**

### **Study design**

We carried out an observational retrospective study among patients living in New Caledonia or Futuna with a biologically confirmed leptospirosis admitted in local health care facilities between January 2007 and December 2009. Outpatients testing results for leptospirosis were not included in the analysis.

Leptospirosis was defined by a compatible clinical syndrome (any combination of chills, myalgia, jaundice, conjunctival suffusion, renal failure, haemorrhage, or pulmonary failure with or without fever) and laboratory confirmation performed at the Institut Pasteur in New Caledonia. All sera were analyzed using the Micro Agglutination Technique at Institut Pasteur in New Caledonia using representative strains of the serogroups known to exist in this region (Berlioz-Arthaud, 2007). Additionally, acute sera were analyzed with the PCR or a real-time PCR used for diagnosis and surveillance at Institut Pasteur in New Caledonia (Picardeau, 2013). Both laboratory tests were routinely performed as part of the diagnosis procedure.

Laboratory analysis used sera collected at the first medical visit. In order to maximize case detection, a more sensitive definition was used for the first episode of infection while a more specific definition was used for the second episode of infection. Confirmed cases during the initial infection were patients with current or recent fever (within a week before presentation), a positive (real-time) PCR, a seroconversion (from nil to >400 in MAT) or a significant rise in serological titers (a 2-fold increase in IgM-ELISA or a 4-fold increase in MAT titers between acute and convalescent sera). A convalescent serum was collected 2 weeks after the initial presentation.

Probable cases were not considered. Reinfection was defined as the development of a new infection with *Leptospira* occurring after successful antimicrobial treatment of a prior episode of leptospirosis. Identification of presumptive serogroup was based on MAT results. Recommended antibiotic therapy was amoxicillin 50mg/kg three times a day for 5 to 7 days for both infection and reinfection.

To assess severity of symptoms during recurrent episode of leptospirosis, clinical and biological characteristics were compared between first and second episode of the disease. A change in exposure risk factors after the first episode of infection was also recorded among patients admitted for reinfection.

## **Results**

During the 3 year-study period, a total of 450 episodes of confirmed leptospirosis were diagnosed both in Futuna (n=153) and New Caledonia (n=297). Five patients experienced a recurrent episode of the disease, including 3 cases in Futuna and 2 cases in New Caledonia. No patient experienced more than one recurrent episode. The mean interval between the first and second episode was 23 months, and never < 12 months. Demographic, clinical and biological characteristics of patients with first and second episode are described in Table 19. All of the recurrent episodes included fever and jaundice, whereas headache, myalgia and conjunctival suffusion were present in 4 cases (80%). During the first episode, renal function impairment, Weil disease and pulmonary involvement were noted in 2 (40%), 1 (20%), and 2 (40%) cases, respectively. In contrast, none of these complications were reported in the second episode of infection. None of the patients were known to be immunosuppressed, to take immunosuppressant medications, or to have diabetes mellitus. **HIV-tests were not systematically performed.**

<b>Demographic characteristics</b>		
<b>Age (median IQR)</b>	36 [24-47]	
<b>Male n(%)</b>	5 (100)	
<b>Oceanian ethnicity n(%)</b>	5 (100)	
	<b>First episode</b>	<b>Second episode</b>
<b>Symptoms n (%)</b>		
<b>Fever (&gt;38°) at admission</b>	5 (100)	5 (100)
<b>Jaundice</b>	5 (100)	4 (80)
<b>Myalgia</b>	4 (80)	4 (80)
<b>Headache</b>	5 (100)	4 (80)
<b>Conjunctival suffusion</b>	4 (80)	4 (80)
<b>Oliguria</b>	3 (60)	0 (0)
<b>Pulmonary involvement (cough, dyspnea, auscultation abnormalities)</b>	2 (40)	0 (0)
<b>Weil's disease</b>	1 (20)	0 (0)
<b>Short delay between onset of symptoms and initiation of antibiotics (&lt;2 days)</b>	<b>2 (40)</b>	<b>3 (60)</b>
<b>Biology mean [95%CI]</b>		
<b>Bilirubin (mg/dL)</b>	41 [34-55]	31 [23-49]
<b>Creatinine (µM/dL)</b>	138 [85-152]	99 [62-140]
<b>Platelet count (Giga/L)</b>	148 [67-175]	204 [157-248]

**Table 19. Demographic, clinical and biological characteristics of patients diagnosed with repeated episodes of leptospirosis in New Caledonia and Futuna, 2007-2009.**

During the initial episode, Icterohaemorrhagiae and Australis serogroup were identified in 3 and 2 cases, respectively. The Icterohaemorrhagiae serogroup was identified in both infection and reinfection in two cases. Among those cases, a four-fold decrease in the anti-leptospital antibody baseline titre from time of previous infection was reported. PCR confirmation was available for all cases among those experiencing recurrent leptospirosis (Table 20).

	Case 1	Case 2	Case 3	Case 4	Case 5
<b>Setting</b>	Futuna	Futuna	Futuna	New Caledonia	New Caledonia
<b>Serogroup (first episode)</b>	Icterohaemorrhagiae	Australis	Icterohaemorrhagiae	Australis	Icterohaemorrhagiae
<b>PCR</b>	positive	not performed	not performed	positive	positive
<b>MAT 1 (on admission)</b>	1:160	1:160	1:320	1:320	1:160
<b>MAT 2 (two weeks)</b>	1:640	1:5120	1:1280	1:640	1:640
<b>Serogroup (second episode)</b>	Icterohaemorrhagiae	Icterohaemorrhagiae	Australis	Icterohaemorrhagiae	Icterohaemorrhagiae
<b>PCR</b>	positive	positive	positive	positive	positive
<b>MAT 1 (admission)</b>	1:160	1:1280	1:640	1:320	1:160
<b>MAT 2 (two weeks)</b>	1:1280	not performed	not performed	1:1280	1:1280

**Table 20. Serogroups characteristics identified in patients diagnosed with repeated episodes of leptospirosis in New Caledonia and Futuna, 2007-2009.**

All of the subjects who experienced leptospirosis received a standard course of antibiotic treatment during their initial and recurrent episodes. The interval between disease onset, admission and treatment was about the same during the first and the second episode of disease (Table 20).

There was no change in exposure risk factors after the first infection among people experiencing recurrent episodes, including time spent outdoors, fresh water exposure, use of personal protection measures, ownership of cattle.

## Discussion

Residents of endemic sites may experience sequential episodes of leptospirosis due to repeated exposure in relation to local lifestyle. Varied serotypes present in floods rather than a lack of capacity to generate single serotype immunity is speculated as a cause of reinfection. However, specific immunity resulting from a previous episode of leptospirosis with the Icterohaemorrhagiae serogroup did not protect against subsequent infection against the same serogroup in two cases presented in this study. This finding is consistent with another study where one third only of infected patients maintained a seroprevalence for the same serogroup (Esteves, 2014). According to these results, use of serology may be useful in the case of presentation with recurrence, at least with Icterohaemorrhagiae serogroup. There are several other potential explanations for reinfection, including living, working, and/or participating in recreation in potentially contaminated environments. Repeated contact with water or soil contaminated by the urine of mammalian reservoirs such as rodents, dogs, cattle and pigs are quite common. Despite receiving oral and written recommendations about how to reduce the risk of contamination, subjects reported new

contacts with potentially contaminated environment. Unexpectedly, patients with recurrent leptospirosis did not seek medical attention sooner than did those who had only a single episode. Whether being immunocompromised increases the risk of reinfection has not been extensively studied.

The acute clinical presentation in people reinfected by the agent of leptospirosis was much like that during the initial episode, although symptoms tended to be less severe during the second episode. This finding is in line with the data reported by Esteves et al. in Portugal (Esteves, 2014). As recently advocated (Brett-Major, 2012), all patients included in this study received antibiotics empirically (amoxicillin or cephalosporin), since they presented early during the course of the disease in both episodes. All cases with reinfection were males, which is in line with usually reported male predominance in leptospirosis. Whether it only reflects higher exposure of the male population through agricultural and farming activities or sex-specific susceptibility factors remains to be determined. A high number of cases in young age classes possibly reflects a frequent exposure of young active men involved in agriculture and farming activities. However, the hypothesis that older age classes are relatively protected by the immunity resulting from previous exposures also deserves consideration.

Measures are available to limit exposure to contaminated environment, including footwear, avoiding swimming in fresh water, and seeking veterinary services. However, compliance with these recommendations is suboptimal, since farm workers are traditionally barefoot and bathing in rivers is refreshing during the hot season. Alternative interventions designed to decrease leptospiral density in domestic animals, such as vaccination, have not been widely adopted for a variety of reasons, including cost.

Strain variability may be a means by which *Leptospira* species circumvent the host's immune response to a previous infection and cause reinfection. Some reinfections are caused by a species of *Leptospira* that is distinctly different from the one that caused the original infection. However, there may also be considerable strain variation within a particular species of *Leptospira*. The role of microbiological variation as a predisposing factor for reinfection deserves further study. The genus *Leptospira* consists of 20 species and includes nine pathogenic, five intermediate and six saprophytic species. There are more than 300 distinct leptospiral serovars recognised and these are arranged in 25 serogroups (Picardeau, 2013). In theory, any parasitic *Leptospira* may infect humans. However, only a small number of serovars will be endemic in any particular setting. The

role of microbiological variation as a predisposing factor for reinfection deserves further study.

This study suffers from several limitations. First, this was a passive case finding scenario done retrospectively with potential screening bias which could have affected capture of both initial and recurrent episodes creating misclassification risks in both directions. The observed incidence rate of reinfection may have been underestimated since this report was limited to symptomatic cases requiring hospital admission, while asymptomatic or subclinical reinfection rate have demonstrated to be high according to prior cohort studies in Brazil among high-risk individuals (Felzemburgh 2014). On the other hand, we cannot exclude that second infections were relapses, in particular for patients with same serogroups on second and first infections. Unfortunately, genotyping data at initial and subsequent infections for positive PCR samples were not available. However, the long delay observed between the two episodes is in favour of reinfection. Second, serological cross-reactivity between serovars and serogroups of *Leptospira* may have led to misclassification. Indeed, infected people with one serovar are likely to have antibodies against the infecting serogroup that cross-react with other serogroups (although usually at a lower level) in the MAT. However, in areas where the serogroups of *Leptospira* present have been well described by isolation studies, serological examination of the infected people strongly suggest the infecting serogroup. In addition, quality control of antigens used in the MAT were regularly performed in the laboratory. Third, people that have been vaccinated against leptospirosis may have antibodies against the serogroups present in the vaccine used. However, none of the patients included in this study were vaccinated. Finally, lack of comparison of exposures and risks among those who did and did not present with reinfection is limiting the assertion that previous immunity confers protection against recurrences.

## **II.12. Conclusion, implications and perspectives for future research**

### **II.12.1. Principal findings**

This thesis explored three neglected clinical aspects of symptomatic leptospirosis in Oceania. More specifically, this work provided epidemiological and clinical data on leptospirosis in children in New Caledonia. In addition, issues raised by this complex infectious disease have been explored in the field of clinical epidemiology, including Jarisch-Herxheimer reaction and reinfections.

There are four principal findings from this series of studies. First, leptospirosis in New Caledonia is responsible for a limited number of admissions among children due to milder symptomatic

clinical presentation. Second, although generally assumed to be a rare event, the true prevalence of JHR in leptospirosis is unknown in most part of the world. The awareness of this event is probably insufficient considering the significantly high proportion of JHR observed in Futuna. *Leptospira interrogans* serogroup Australis and short delays between the onset of symptoms and the initiation of antibiotherapy are associated with an increased risk for JHR in New Caledonia and Futuna. Third, recurrent episodes of symptomatic leptospirosis appear to be non-severe in those settings. Finally, specific immunity resulting from a previous episode of leptospirosis with the serogroup *Icterohaemorrhagiae* did not protect against subsequent infection against the same serogroup in two cases.

### **II.12.2. Implications for policy and practice**

Leptospirosis remains a public health threat, including in the younger age groups with potential atypical clinical presentation. To prevent and control this public health threat in children, policies and interventions must cut across sectors and disciplines. Given the disease's important links to climate, environment and the complex animal-host dynamics, interventions addressing leptospirosis should involve all relevant sectors. Additionally, approaches taken to control leptospirosis may help control other zoonotic diseases and thus improve cost effectiveness - for example, rodent-control efforts used for leptospirosis may affect the incidence of other rodent-borne diseases. Leptospirosis provides the opportunity to evaluate One Health strategies for prevention and control. The lack of recognition of leptospirosis as an important zoonotic disease had previously hampered consideration of such approaches. Additional research is needed to quantify the burden of leptospirosis among children, which incorporates an assessment of its impact on school attendance.

Clinicians should be aware of JHR as a potential complication of leptospirosis in Oceania. All leptospirosis guidelines and local leptospirosis protocols should stress on systematic monitoring for clinical status early after antibiotic administration to detect occurrence of JHR. The JHR is a self-limiting reaction, the treatment of which is predominantly supportive. For the time being, its occurrence cannot be prevented, and therefore, it should be anticipated in all patients receiving antibiotic treatment for leptospirosis and the course of antibiotic treatment started initially must be fully completed. It is important to note that the occurrence of a JHR, at times, may point toward an undiagnosed leptospirosis in a patient receiving antibiotic treatment for another suspected disease.

### **II.12.3. Implications for future research**

Identifying pathogenesis mechanisms explaining heterogeneity in clinical manifestations of leptospirosis remains one of the most significant research challenges in the field. Interactions between the endocrine, nervous and immune systems undoubtedly influence the outcome leptospirosis as in most of other infections. Physiologic and immunologic factors associated with improved paediatric outcomes require further investigation to provide insights into the pathogenesis of severe leptospirosis. Later in life very probably due to socioeconomic behavior, such as higher pathogen exposure during agricultural or occupational activities, men are more susceptible to many infections, including leptospirosis. Contrary to human schistosomiasis, acquired immunity through years of exposure to spirochaetal antigens does not seem to be applicable to leptospirosis. Frequency and intensity of exposure does not explain entirely the observed variation in clinical or biological presentations. Other age-related factors than level of exposure such as hormones might account for the differences. First, puberty-related hormones, such as dehydro-epiandrosterone or testosterone could be involved in shaping age-intensity profiles in endemic communities. In addition, involution of the thymus decreases the output of native T cells, and antigen-experienced T cells accumulate constricting the T-cell repertoire. Highly differentiated effector T cells produce more pro-inflammatory cytokines, which together with activated innate immune cells contribute to a systemic pro-inflammatory milieu in older age. Improving the understanding of the potential relationship among leptospirosis and pubertal hormone levels and changing of T cells profile may provide a paradigm explaining host-related response to leptospirosis accounting for the epidemiology of infection. Second, sex steroid hormones are thought to contribute to differences in humoral and cellular responses to infection and vaccination in men and women. Notably, macrophages treated in vitro with estradiol showed decreased secretion of the proinflammatory cytokines interleukin (IL) IL-1, IL-6, and tumor necrosis factor (TNF- $\alpha$ ). Estrogens also exhibit indirect effects on the immune system by modulating the levels of growth hormone, prolactin, or thymosin. The general paradigm on sex steroid hormones influencing the immune system stipulates that estrogens have immune-enhancing effects. In contrast, progesterone and androgens such as testosterone exert mainly immunosuppressive properties. Studying influence of sex steroid hormones on leptospirosis may help to understand variation in individual susceptibility to the infection.

Individual and environmental factors associated with JHR occurrence and severity deserve attention for future research. In humans, JHR has been shown to be associated with host production of inflammatory mediators and anti-inflammatory cytokines. Large well-designed

studies are required to precise the true incidence of JHR based on valid biological markers, including cytokines and lipoproteins. Similarly, strain-related factors associated with JHR occurrence and its impact on outcome remain to be explored. Whether a particular class of antibiotics influences the occurrence of the reaction, remains unclear. Penicillin treatment through inhibition of bacterial cell wall synthesis makes spirochetes more susceptible to phagocytosis, which then stimulates cytokine release, including TNF- $\alpha$  and IL-6 and IL-8. Other antibiotics should be further studied in the treatment of leptospirosis. For example, azithromycin exerts bactericidal effects through inhibition of protein synthesis. Furthermore, azithromycin have long been recognized to exert immunomodulatory and anti-inflammatory actions. Some data suggest that azithromycin suppresses the production of inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and macrophage inflammatory protein 2 apart from the antibiotic effect. In addition, attempts at modulating the inflammatory response to anti-leptospirosis treatment may alleviate the risk. Agents that may help prevent or ameliorate the JHR are either not available in routine practice or not entirely effective. Newer preventative strategies such as humanized-monoclonal antibody (HuScFv) hold promise especially in patients with drug hypersensitivity, but this treatment should be further studied.

The Jarisch-Herxheimer reaction in pregnant women has been associated with early spontaneous termination of pregnancy and premature labour when using penicillins in syphilis. Little is known on leptospirosis with JHR during pregnancy. This specific context warrants consideration to provide information on materno-fetal morbidity and improve outcome and prevention. More sustainable practices considering ecosystems are needed to strengthen disease control in vulnerable population, such as pregnant women. Leptospirosis is a social-ecological problem, which often occurs in the context of social inequity. Therefore there is a critical need to evaluate and address the investment case for interventions that target the underlying environmental conditions and infrastructure deficiencies, such as health access, in order to make sustainable progress against this neglected disease.

Finally, individual immunological factors should be scrutinized to explain the observed low incidence of symptomatic reinfection. Since only a small number of serovars will be endemic in any particular setting, the role of microbiological variation as a predisposing factor for reinfection merits further studies. Such results would certainly benefit to vaccine development research, by completing knowledge of mechanisms of protective immunity against leptospiral infection. One limitation that hinders the development of vaccines against leptospirosis is the lack of correlates of

protection, immune markers that have contributed to many vaccinology studies. Both humoral and cellular immune responses have been reported to play roles in the response against leptospirosis. Long-term efficacy studies of antileptospiral vaccines have not been published. So far, killed bacterial vaccines have only short-term efficacy and incomplete protection, necessitating repeated vaccination to maintain immunity with the attendant side-effect profiles that might be expected. Vaccines for leptospirosis are routinely used in livestock and domestic animals, although they do not appear to be transmission-blocking. Investment towards identifying interventions, such as vaccines, may yield synergistic health and societal benefits for poor populations in developing countries.

### **II.13. Abstract**

Leptospirosis is an endemo-epidemic zoonotic disease associated with potentially fatal renal, cardiovascular or pulmonary failure. Several aspects of the disease are poorly explored, including infections in children, Jarisch-Herxheimer reactions (JHR) and symptomatic re-infections. The objectives of the studies were the following: to describe disease spectrum and outcome differences in children and adolescents admitted for leptospirosis in a large at-risk population; to quantify frequency of and risk factors for JHR in leptospirosis management; and to assess incidence and characteristics of reinfection by leptospirosis in the Pacific region. Using a retrospective data collection, clinical and laboratory data were obtained on hospitalized confirmed cases in New Caledonia and in Futuna. A case-control study was designed to identify risk factors using a logistic regression model. There are four principal findings from this series of studies with direct implications for clinical practice in those settings. First, leptospirosis in New Caledonia is responsible for a limited number of admissions among children due to milder symptomatic clinical presentation. Second, although generally assumed to be a rare event, the true prevalence of JHR in leptospirosis is unknown in most part of the world. The awareness of this event is probably insufficient considering the significantly high proportion of JHR observed in Futuna. Third, recurrent episodes of symptomatic leptospirosis appear to be non-severe. Finally, specific immunity resulting from a previous episode of leptospirosis with the serovar Copenhageni did not protect against subsequent infection against the same serovar. Future research based those results may provide insights into the still challenging pathogenesis of this complex infectious disease.

### **II.14. Résumé en français**

#### **Introduction**

La leptospirose, spirochetose tropicale négligée, est considérée comme la zoonose la plus répandue

dans le monde. Selon l'Organisation Mondiale de la Santé, les formes sévères représentent 5 à 15% des infections humaines et le taux de létalité peut varier entre 5% et 30%. En Océanie et particulièrement en Nouvelle-Calédonie et à Futuna, la leptospirose est une préoccupation majeure de santé publique: son incidence annuelle moyenne est de 45 pour 100 000 habitants et peut atteindre 150 pour 100 000 habitants pendant les saisons pluvieuses. Malgré les progrès considérables accomplis pour comprendre la physiopathologie de la maladie, plusieurs aspects de la leptospirose encore négligés méritent d'être explorés: les infections symptomatiques pédiatriques, la réaction de Jarisch-Herxheimer (JH) pourtant bien étudiée dans les autres spirochetoses, et les reinfections nécessitant une hospitalisation.

### **Questions posées et objectifs**

La pratique clinique suggère que les enfants hospitalisés pour leptospirose sont moins sévèrement atteints que les adultes. Les raisons susceptibles d'expliquer cette différence de présentation sont peu claires. L'objectif de l'étude 1 était de décrire l'épidémiologie et le profil clinico-biologique des formes pédiatriques de leptospirose nécessitant une hospitalisation en Nouvelle Calédonie et d'identifier des différences en fonction des classes d'âge.

La réalité de la réaction de Jarisch-Herxheimer – bien connue dans les autres spirochetoses – reste un sujet controversé dans la leptospirose. Afin d'effectuer une mise au point sur le sujet, une revue systématique de la littérature préalable à été réalisée (Etude 2) avant de préciser la prévalence, la sévérité, et les facteurs de risque des réactions de Jarisch-Herxheimer au cours des épisodes de leptospirose humaine traitée par antibiotiques en Nouvelle Calédonie et à Futuna (Etude 3).

Le degré d'immunoprotection conférée par un premier épisode de leptospirose contre les épisodes de ré-infections par un serovar identique ou non est mal connu chez l'homme. Des cas avérés de nouvelle infection par un serovar identique ont été observés en Nouvelle Calédonie et à Futuna. L'étude 3 à été menée afin de mesurer l'incidence et préciser les caractéristiques cliniques des épisodes de ré-infection de leptospirose humaine nécessitant une hospitalisation en Nouvelle Calédonie et à Futuna.

### **Matériels et méthodes**

#### **Données socio-démographiques, économiques et sanitaires en Nouvelle Calédonie et à Futuna**

La Nouvelle Calédonie est un territoire d'outre-mer français situé dans la région Pacifique Sud. Sa population qui comportait 268 767 habitants en 2014 est une mosaïque ethnique (45 % de

mélanésiens, 22 % d'européens, 11% de wallisiens ou de futuniens, 1% d'asiatiques, 13% de métis, et 8% d'autres origines ethniques). Il s'agit d'un pays à revenus élevé mais avec une distribution des richesses inégale en fonction des ethnies. Les provinces Nord et des Îles Loyauté, qui comptent une population majoritairement mélanésienne, ont un revenu médian 2 à 2,5 fois moindre par rapport à la Province Sud où réside une majorité d'européens. La densité médicale en Nouvelle-Calédonie est de 223 médecins pour 100 000 habitants. Il existe une disparité importante entre le Nord et le Sud avec une densité médicale de 274/100 000 dans le Sud contre 96/100 000 dans le nord et 80/100 000 dans les îles Loyauté. Le Centre Hospitalier Territorial de Nouvelle Calédonie (CHT) est le seul centre tertiaire de l'archipel pourvoyeur de soins spécialisés sur le territoire. L'accès aux soins de proximité est assuré par des centres médico-sociaux dans chacune des communes.

Futuna est un archipel tropical de petite superficie (64 km<sup>2</sup>), au relief montagneux, situé environ 2000 km au nord-est de la Nouvelle-Calédonie. Les 3.612 habitants recensés en 2013 vivent dans 14 villages répartis le long de la côte. L'économie repose sur l'agriculture de subsistance traditionnelle: environ 80% de la population active est impliquée dans la culture de noix de coco et de légumes, l'élevage de bétail essentiellement porcin et de la pêche. Les revenus proviennent de subventions du gouvernement français, les licences de droits de pêche accordées au Japon et à la République de Corée, les taxes à l'importation, et les envois de fonds des travailleurs émigrés en Nouvelle-Calédonie. Futuna possède un hôpital de 15 lits et deux dispensaires. Tous les traitements sont gratuits, y compris les soins aux patients hospitalisés. L'incidence annuelle de la leptospirose dans l'île a été particulièrement élevée de 2005 à 2009 avec une moyenne de 1085 cas pour 100 000 au cours de cette période.

### **Definitions**

Le diagnostic de leptospirose reposait sur l'association d'une présentation clinique compatible (fièvre, frissons, myalgies, ictère, suffusion conjonctivale, défaillance rénale, alvéolite hémorragique) et une confirmation biologique avec au moins une des caractéristiques suivantes: 1) test d'agglutination microscopique positif (en utilisant un panel de 12 serovars) et un titre d'agglutines > 1:800 en phase aiguë, 2) mise en évidence d'une séroconversion entre deux prélèvements effectués respectivement en phase aiguë et en phase de convalescence, 3) une augmentation de quatre fois le titre des agglutines entre deux prélèvements, ou 4) un résultat PCR positif.

La réaction de JH était définie par la combinaison d'une apparition brutale d'une fièvre, de frissons, avec ou sans variation des paramètres hémodynamiques ou respiratoires dans un délai de 6 heures après la première administration des antibiotiques pour traiter un cas suspect de leptospirose et une résolution des symptômes dans les 24 heures suivantes.

### **Matériel et méthodes**

Tous les patients hospitalisés en Nouvelle Calédonie ou à Futuna avec une leptospirose biologiquement confirmée ont été inclus sur des périodes différentes selon les études dans un intervalle de temps compris entre janvier 2006 et décembre 2012. Une étude de type cas-témoin a été réalisée pour identifier les facteurs de risque de réaction JH. Les cas étaient les patients hospitalisés pour suspicion de leptospirose secondairement confirmée présentant une réaction de JH. Le groupe témoin était constitué des patients hospitalisés pour leptospirose confirmée par diagnostic biologique mais ne présentant pas de réaction de JH. Les données épidémiologiques, cliniques, biologiques et moléculaires ont été recueillies de manière rétrospective pour l'ensemble des études.

### **Statistiques**

Les variables descriptives sont présentées avec leur moyenne  $\pm$  déviation standard (DS) ou médiane et intervalle interquartile. Les variables discontinues ont été comparées par le test du Chi-2. La comparaison des variables continues a été réalisée par le test t de Student. Lorsque la fréquence des événements était inférieure à 5 ou que les variables ne présentaient pas de distribution normale, le test exact de Fisher et le test de Mann-Whitney ont été utilisés respectivement pour les variables discrètes et continues. Les calculs de prévalence ont été effectués avec un intervalle de confiance (IC) de 95%. Les facteurs de risque de réaction de JH ont été identifiés par régression logistique en utilisant un modèle de Cox univarié puis multivarié. Un seuil  $p < 0,05$  a été considéré comme significatif. Le logiciel de statistiques STATA 11 a été utilisé pour réaliser l'ensemble des analyses.

### **Considérations éthiques**

Les trois études ont été approuvées par le Comité d'éthique du Centre Hospitalier Territorial. Compte tenu du caractère rétrospectif, observationnel et anonymisé des études, le consentement éclairé signé n'était pas requis de la part des patients. Tous les patients ont néanmoins donné leur accord oral après avoir été informés des objectifs de chaque étude.

## **Étude 1: Association entre l'âge et de la sévérité de la leptospirose chez les enfants**

### **Contexte**

En zone endémique, la leptospirose semble plus fréquente et plus sévère chez les adultes que chez les enfants. Peu de données sont disponibles pour expliquer les raisons de cette différence qui restent imprécises. L'objectif de l'étude était de décrire et comparer la présentation clinico-biologique et le devenir de la maladie chez les enfants et les adolescents hospitalisés pour leptospirose dans une population exposée.

### **Méthodes**

Il s'agit d'une étude observationnelle analytique rétrospective. Les données cliniques et de laboratoire ont été obtenues sur les cas hospitalisés en Nouvelle-Calédonie sur une période de 7 ans (2006-2012).

### **Résultats**

Les données de 60 patients <18 ans (25 enfants de moins de 14 ans et 35 adolescents âgés de 14 à 17 ans) hospitalisés pour leptospirose ont été analysées. Comparés aux enfants, les adolescents présentaient plus souvent les caractéristiques de la maladie de Weil ( $p = 0,02$ ), incluant une défaillance hépatique et rénale avec ou sans alveolite hémorragique. Les réactions de Jarisch-Herxheimer ont été observées plus souvent chez les adolescents ( $p < 0,01$ ). Le taux de létalité observé était faible (1 adolescent versus 0 enfant).

### **Limites de l'étude**

Plusieurs limites de cette étude doivent être soulignées. D'abord, le caractère rétrospectif de la collecte des données n'a pas permis de retrouver un certain nombre de dossiers de patients qui ont dû être secondairement exclus de l'analyse. Ensuite, l'identification des serovars infectants n'ont été possible que dans un nombre limité de cas. De plus, des données cliniques importantes telles que la présence de signes de puberté n'était pas disponible dans les dossiers. Enfin, les critères d'hospitalisation pour les enfants infectés par la leptospirose diffèrent de ceux des adolescents dont certaines formes pauci-symptomatiques ont pu être traitée hors de l'hôpital.

### **Conclusion**

Les enfants pré-pubères hospitalisés pour leptospirose ont des atteintes moins sévères que les adolescents qui présentent un profil clinico-biologique comparable à celui des adultes. D'autres études sont nécessaires pour explorer les facteurs de sévérité liés à l'hôte et dépendants de l'âge, y compris les changements physiologiques qui accompagnent à la puberté.

## **Étude 2: Revue systématique de la littérature sur la réaction de Jarisch-Herxheimer dans la leptospirose**

## **Contexte**

La leptospirose est une maladie zoonotique endémo-épidémique potentiellement mortelle en cas de défaillance rénale, cardiovasculaire ou respiratoire. Le traitement recommandé repose sur l'administration d'antibiotiques, qui peuvent induire une réaction de JH. Peu d'informations sont disponibles concernant l'importance de cet événement indésirable dans la leptospirose. Afin de quantifier la fréquence et l'impact des réactions de JH au cours de la leptospirose, nous avons effectué une revue systématique de la littérature sur le sujet.

## **Méthodologie**

Tous les articles incluant des patients traités pour leptospirose, les recommandations nationales et internationales ainsi que les ouvrages universitaires de référence et les rapports techniques de l'Organisation Mondiale de la Santé sur le sujet ont été recensés et examinés.

## **Principaux résultats**

Les publications décrivant les réactions de JH dans la leptospirose sont très limitées en nombre et sont composées principalement de description de cas clinique unique et de petites séries de cas. Le seul essai randomisé contrôlé dont l'objectif principal était d'évaluer l'incidence des réactions de JH était de faible puissance statistique. Peu de recommandations sur la prise en charge de la leptospirose s'intéressent aux réactions de JH qui peuvent pourtant s'avérer fatale.

## **Limites de l'étude**

Il faut admettre un certains nombres d'imperfections méthodologiques à cette revue. En effet, on ne peut exclure un biais de publication. De plus, des rapports non publiés ou non référencés dans les bases de données explorées ont pu échapper à ce recensement. Par ailleurs, les patients présentant des co-infections non dépistées ont pu être inclus. Enfin et surtout, le diagnostic de réaction de JH ne reposait sur aucun dosage biologique objectif et la définition clinique de l'évènement n'était pas homogène entre les études conduisant à un possible biais de classification.

## **Conclusions**

Supposé être un événement rare, la fréquence des réactions de JH dans la leptospirose est mal connue. La conscience collective de cette complication est insuffisante dans le monde de l'infectiologie. Le suivi précoce de l'état clinique après l'administration d'antibiotiques devrait être systématique. De larges études bien conduites sont nécessaires pour préciser l'incidence, la sévérité, l'impact, et les facteurs déclenchants des réactions de JH.

## **Étude 3: Incidence et facteurs de risque des réactions de Jarisch-Herxheimer chez les patients atteints de leptospirose**

### **Contexte**

L'administration d'antibiotiques chez les patients infectés par une spirochètose peut déclencher une réaction de JH. L'importance de cette réaction dans la leptospirose reste mal définie, en particulier en Océanie.

### **Méthodes**

Il s'agit d'une étude observationnelle rétrospective de type cas-témoin. L'incidence et les facteurs de risque des réactions de JH ont été étudiés rétrospectivement entre 262 patients atteints de leptospirose biologiquement confirmée et ayant reçu un traitement par amoxicilline en Nouvelle-Calédonie et à Futuna. Les cas étaient définis par les patients présentant une réaction de JH et les témoins étaient définies par les patients ne présentant pas de réaction de JH.

### **Résultats**

La prévalence de réactions de JH était de 21% (12% en Nouvelle-Calédonie et 44% à Futuna). Deux facteurs de risque étaient indépendamment associés à la survenue de réactions de JH occurrence: le sérovar Australis comme souche infectante (OR = 2,60 [IC 1,40 à 5,62]) et un délai court (<3 jours) entre l'apparition des symptômes et le début de l'antibiothérapie (OR = 2,14 [IC 1,11 à 4,38]).

### **Limites de l'étude**

Les résultats de cette étude doivent être tempérés par des carences majeures d'ordre méthodologique. Premièrement, il existe un biais de classification inhérent au caractère rétrospectif de l'étude qui concerne notamment les patients avec une évolution rapidement favorable ou au contraire qui présente un sepsis sévère initialement compensé. Deuxièmement, la définition de la réaction de JH était uniquement clinique sans confirmation par des dosages de marqueurs biologiques fiables. Ainsi, certains patients ont pu faire l'objet d'un biais de classification, en particulier ceux dont l'évolution était marquée par une aggravation clinique malgré une antibiothérapie bien conduite. De plus, il n'est pas exclu que la présence de fièvre ou de frissons était contemporaine du crépuscule pharmacodynamique des antipyrétiques. Troisièmement, la taille restreinte de l'échantillon de l'étude limitait l'analyse en sous-groupe afin d'étudier certains facteurs de risque individuels tel que l'origine ethnique. Quatrièmement, la technique de microagglutination pour l'identification du sérovar infectant possède une spécificité relativement faible. Enfin, certains facteurs confondants liés à l'hôte, au pathogène ou à l'environnement et non pris en compte dans l'analyse pourraient limiter la fiabilité des résultats qui suggère une association entre sérovar infectant et réaction de JH.

### **Conclusions**

Les cliniciens devraient être conscients de la possibilité de survenue de réactions de JH comme complication précoce potentielle de la leptospirose. Les facteurs intimes liés à la souche infectante

et l'impact des réactions de JH sur le devenir des patients restent à explorer.

#### **Étude 4: Réinfection par la leptospirose: suivi rétrospectif longitudinal sur une période de 3 ans dans deux îles du Pacifique**

##### **Contexte**

Les épisodes récurrents de leptospirose sont peu décrits dans la littérature. L'objectif de l'étude était d'évaluer l'incidence et les caractéristiques des épisodes de réinfections par la leptospirose dans une large population à risque dans deux îles du Pacifique sud.

##### **Méthodes**

Il s'agit d'un suivi de cohorte observationnel rétrospectif. Tous les patients admis pour leptospirose confirmée ont été suivis sur une période de 3 ans en Nouvelle-Calédonie et l'île de Futuna (2007-2009). Tous les épisodes de réinfections symptomatiques nécessitant une hospitalisation ont été enregistrés.

##### **Résultats**

Parmi les 446 personnes admises pour leptospirose confirmée, 5 cas de réinfection ont été identifiés en Nouvelle-Calédonie (2 cas) et à Futuna (3 cas) au cours de la période de suivi. Tous les épisodes récurrents sont survenus chez les adultes qui présentaient de la fièvre (100%), un ictère (80%), une suffusion conjonctivale (80%), des céphalées (80%), et des myalgies (80%). Une insuffisance rénale a été rapportée dans 2 cas. Aucun syndrome de Weil ni de décès n'ont été observés. Le temps moyen écoulé entre les deux épisodes était de 23 mois. Le sérotype a été identifié dans tous les cas récurrents et était similaire à l'épisode précédent de 2 (40%) cas.

##### **Limites de l'étude**

Plusieurs insuffisances méthodologiques méritent d'être mentionnées. Dans la mesure où l'inclusion des cas de leptospirose s'est faite de manière rétrospective, un biais de classification n'est pas exclu à la fois pour les épisodes initiaux et les épisodes récurrents. Par ailleurs, une sous-estimation de l'incidence observée des ré-infections est plausible compte tenu du fait que seuls les cas symptomatiques ayant nécessité une hospitalisation ont été inclus dans cette étude excluant ainsi les épisodes de ré-infections asymptomatiques ou paucisymptomatiques qui sont pourtant fréquents dans d'autres régions du monde, notamment au Brésil. Ensuite, les réactions croisées serologiques entre sérotypes et serovars a sans doute conduit à un biais de classification. En effet, les personnes infectées par un sérotype développent des anticorps potentiellement détectés par des réactifs correspondant à des sérotypes différents en utilisant la technique de microagglutination. Ce biais de classification a sans doute été limité par les contrôles de qualité régulièrement effectués dans le laboratoire ayant effectué les analyses. Il est peu probable que

patients inclus dans l'étude aient développés des anticorps anti-leptospires par le biais de la vaccination dans la mesure où cette pratique de prévention chez les humains n'est pas utilisée en Nouvelle Calédonie ou à Futuna. Enfin, aucune comparaison des risques d'infection et des facteurs d'exposition n'a été réalisée entre les patients qui présentaient une ré-infection et ceux qui n'en présentaient pas. Ceci limite les conclusions concernant la protection potentielle de certains serogroupes contre d'éventuelles ré-infections.

### **Conclusion**

Les épisodes récurrents de leptospirose symptomatique semblent être non-sévères, probablement en raison d'une sensibilisation préalable de la communauté médicale qui traite précocement les cas suspects. Le rôle potentiel des facteurs immunologiques individuels dans la physiopathologie de la maladie pourrait expliquer la faible incidence de réinfections symptomatiques observée dans cette étude.

### **Principaux résultats**

Cette thèse d'épidémiologie clinique a exploré trois aspects négligés d'une maladie infectieuse complexe: la leptospirose chez les enfants, la réaction de Jarisch-Herxheimer et les épisodes de réinfection par la leptospirose dans deux îles en Océanie. Quatre résultats principaux de cette série d'études sont à souligner. Tout d'abord, la leptospirose en Nouvelle-Calédonie est responsable d'un nombre limité d'admissions chez les enfants en raison de la présentation clinique des symptômes moins sévères. Deuxièmement, bien que généralement supposé être un événement rare, la prévalence réelle des réactions de JH dans leptospirose est inconnue dans la plupart des régions du monde. La prise de conscience de cet événement est probablement insuffisante compte tenu de la forte proportion significative des réactions de JH observée à Futuna. *Leptospira interrogans* séro-groupe Australis et des délais courts entre le début des symptômes et le démarrage de l'antibiothérapie sont associés à un risque accru de réaction de JH en Nouvelle-Calédonie et à Futuna. Troisièmement, les épisodes récurrents de leptospirose symptomatique semblent être peu sévères dans cet environnement. Enfin, l'immunité spécifique résultant d'un épisode de leptospirose avec le sérovar Copenhageni ne semble pas assurer de protection pas contre une nouvelle infection par le même sérovar.

### **Implications pour la recherche**

L'identification des mécanismes pathogénétiques susceptibles d'expliquer l'hétérogénéité des manifestations cliniques de la leptospirose demeure l'un des défis pour la recherche dans ce domaine. Les facteurs physiologiques et immunologiques associés aux formes pédiatriques

souvent peu sévères nécessitent une exploration approfondie pour fournir des pistes explicatives des infections évoluant vers les formes graves. La puberté pourrait avoir un impact sur la gravité rapportée dans les groupes plus âgés. Les changements physiologiques et notamment hormonaux associés à cette phase de transition méritent d'être étudiés plus avant. Les facteurs liés à la nature de la souche infectante comme facteur de risque de réactions de JH restent à être explorés. Par ailleurs, l'influence de la classe d'antibiotique administrée sur la survenue de la réaction n'est pas connue. Des études prospectives à large effectif sont nécessaires pour préciser les facteurs de risque, l'incidence et l'impact des réactions de JH dans la leptospirose. Enfin, les facteurs immunologiques individuels doivent être examinés pour expliquer la faible incidence de réinfection symptomatique observée. Le nombre de sérovars endémiques dans un contexte particulier étant limité, le rôle de la variation microbiologique comme facteur prédisposant aux réinfections mérite de faire l'objet d'autres études. Enfin, ces résultats pourraient certainement bénéficier à la recherche sur le développement de vaccins, en complétant la connaissance des mécanismes de l'immunité protectrice contre l'infection des leptospires. Les études consacrées à l'efficacité à long terme des vaccins anti-leptospire ne sont pas disponibles. Jusqu'à présent, les vaccins bactériens tués ont montré une efficacité à court terme et une protection incomplète, nécessitant des vaccinations répétées pour maintenir une immunité satisfaisante au prix d'effets secondaires attendus.

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# Association between Age and Severity to Leptospirosis in Children

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## Abstract

**Background:** In endemic areas, leptospirosis is more common and more severe in adults compared with children. Reasons to explain this discrepancy remain unclear and limited data focusing on adolescents are available. The objective of the study was to describe disease spectrum and outcome differences in children and adolescents admitted for leptospirosis in a large at-risk population.

**Methods:** Clinical and laboratory data were obtained on hospitalized cases in New Caledonia from 2006 to 2012.

**Results:** Data of 60 patients <18 years of age (25 children under 14 and 35 adolescents aged 14 to 17) with confirmed leptospirosis were analyzed. Compared with children, adolescents presented more often with classic features of Weil disease ( $p=0.02$ ), combining hepatic and renal involvement with or without pulmonary participation. Jarisch-Herxheimer reactions were observed more often among adolescents ( $p<0.01$ ). The overall case fatality rate was low (1 adolescent versus 0 children).

**Conclusion:** Severe leptospirosis in adolescents may be more likely to show adults' characteristics compared with children. Further studies are required to explore age-dependant host factors, including puberty-related physiological changes.

**Citation:** Guerrier G, Hie P, Gourinat A-C, Huguon E, Polfrit Y, et al. (2013) Association between Age and Severity to Leptospirosis in Children. PLoS Negl Trop Dis 7(9): e2436. doi:10.1371/journal.pntd.0002436

**Editor:** Jessica N. Ricaldi, Universidad Peruana Cayetano Heredia, Peru

**Received:** May 16, 2013; **Accepted:** August 9, 2013; **Published:** September 26, 2013

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**Funding:** The authors received no specific funding for this study.

**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

Leptospirosis is an important zoonosis of worldwide distribution caused by pathogenic spirochaetes of the genus *Leptospira*. Humans usually become infected through contact with water or soil contaminated by the urine of mammalian reservoirs such as rodents, dogs, cattle and pigs [1].

Infection most commonly results in asymptomatic or self-resolving illness both in adults and children [2]. In severe cases requiring hospitalization, the disease is however potentially fatal classically presenting with jaundice and renal dysfunction (Weil disease) with or without pulmonary hemorrhagic manifestations. According to estimates from the World Health Organization, more than 500,000 severe cases occur every year worldwide, mostly in tropical and sub-tropical regions.

Along with experienced clinicians' beliefs, several studies suggest leptospirosis to produce more severe presentation in adults compared with children [3,4,5,6,7]. Pathogen as well as host-related factors are believed to play a role in the development of severe leptospirosis in adults [1,8]. However, factors responsible for the milder presentation among children remain unclear. There is limited information available about symptomatic leptospirosis in the under 18 age group in the Pacific region.

New Caledonia is an overseas French-administered territory located in the South Pacific and located 1500 kilometres East of Australia. According to the 2009 census, the population is 245 530 inhabitants with a mean increase of 1.7% per year since 1996 [9]. The population has good access to the health care system of European standards. Climate in New Caledonia is marked by a cool and dry season (from June to September) and a warm and wet one (from December to March). Leptospirosis is endemic to New Caledonia, and is a leading cause of hospital admission during the rainy season [10,11]. From 2006 to 2009, the average annual incidence was 45 cases per 100,000 inhabitants but reached 150 per 100,000 inhabitants during the rainiest months.

The objective of the study was to describe disease spectrum and outcome differences in children and adolescents admitted for laboratory-confirmed leptospirosis in a large at-risk population. We hypothesized that adolescents were more likely to present with all the classic features of Weil disease compared with younger children.

## Methods

### Study design

We carried out an observational retrospective study among patients aged under 18 years old with a biologically confirmed

# The Jarisch-Herxheimer Reaction in Leptospirosis: A Systematic Review

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## Abstract

**Background:** Leptospirosis is an endemo-epidemic zoonotic disease associated with potentially fatal renal, cardiovascular or pulmonary failure. Recommended treatment includes antibiotics, which may induce a Jarisch-Herxheimer reaction (JHR). Since little information on the importance of this adverse event is available, we performed this review to quantify frequency and impact of JHR in leptospirosis management.

**Methodology/Principal Findings:** This review systematically summarizes the literature on the JHR in leptospirosis. To approach the broader aspects of the subject, articles considering the treatment of leptospirosis, national leptospirosis guidelines and textbook and technical reports of the World Health Organisation were reviewed. Publications describing JHR in leptospirosis are very limited and consist mainly of single case reports and small case series. A single randomized control trial specifically assessed the JHR occurrence, but it has never been systematically investigated in large trials. Not all guidelines and not all literature on leptospirosis mention this reaction which can be fatal.

**Conclusions/Significance:** Although generally assumed to be a rare event, the true prevalence of JHR in leptospirosis is unknown and the awareness of this event is insufficient. All leptospirosis guidelines and local leptospirosis protocols should stress on systematic monitoring for clinical status early after antibiotic administration. Large well designed studies are required to precise the incidence and the impact of JHR as well as the severity and rates between various antibiotics.

**Citation:** Guerrier G, D'Ortenzio E (2013) The Jarisch-Herxheimer Reaction in Leptospirosis: A Systematic Review. PLoS ONE 8(3): e59266. doi:10.1371/journal.pone.0059266

**Editor:** Odir A. Dellagostin, Federal University of Pelotas, Brazil

**Received:** December 17, 2012; **Accepted:** February 13, 2013; **Published:** March 26, 2013

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**Funding:** Institut Pasteur de Nouvelle Calédonie funded this work. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

Leptospirosis is a widespread zoonotic disease of worldwide distribution caused by pathogenic *Leptospira*, a spirochetal organism that is transmitted to humans by exposure to urine of infected mammalian reservoirs such as rodents or wild and domestic animals [1]. The disease has a wide-ranging clinical spectrum from asymptomatic forms to severe presentations. The latter are estimated to occur in 5–15% of all human infections and commonly combine jaundice, renal failure, myocarditis and/or haemorrhage [2] with a significant mortality rate. While a single experimental study demonstrated the relevance of antibiotic administration even in late stages of leptospirosis [3] the benefit of antibiotic therapy on mortality lacks of clinical evidence [4]. However, the current treatment guidelines still rely on antibiotic administration regardless of stage or severity of the disease [5]. Initiation of chemotherapy in spirochetal diseases may precipitate a febrile inflammatory reaction [6,7], known as the Jarisch-Herxheimer reaction (JHR), originally described in patients with syphilis receiving mercury treatment [8,9]. This reaction is characterised by an acute inflammatory response associated with the release of large amounts of cytokines, resulting from clearance

of spirochetes from the circulation [6,7]. Prevalence, clinical manifestations and outcome of JHR have been well studied in syphilis [10], Lyme disease [11], tick-born relapsing fever [12] and louse-born relapsing fever [13–15]. However, a clear idea of importance JHR in leptospirosis is lacking. In order to quantify frequency and impact of JHR in leptospirosis, we systematically reviewed the published literature and put it in a broader perspective, identifying data gaps that should be addressed.

## Methods

### Search Strategy and Selection Criteria

The inclusion criteria were as follows: all publications on leptospirosis treated with antibiotics providing information about the occurrence of early adverse events including JHR were selected. When the original article was not obtainable but the abstract containing the requested information was, the publication was included in the analysis. To avoid multiple counting (duplication) of identical procedures and cases, follow-up publications on identical procedures and cases were traced and excluded. The electronic database PubMed was searched with the keywords “leptospirosis” and “Leptospira”. A second search was performed



**Jarisch-Herxheimer reaction among patients with leptospirosis: incidence and risk factors**

Journal:	<i>American Journal of Tropical Medicine &amp; Hygiene</i>
Manuscript ID:	AJTMH-16-0457
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	08-Jun-2016
Complete List of Authors:	Guerrier, Gilles; Hopital Cochin, Anesthésie-réanimation Lefèvre, Patrick; Service de médecine, Centre Hospitalier du Nord, Koumac, New Caledonia, Medicine Chouvin, Chantal; HINARI Wallis and Futuna, Dispensaire D'Ortenzio, Eric; Institut de Veille Sanitaire, Cire Océan Indien
Key Words:	Leptospirosis, Bacterial Diseases, Clinical Studies, Emerging Diseases

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## TRSTMH

### Reinfection in leptospirosis: a 3-year retrospective study in two Pacific islands

--Manuscript Draft--

<b>Article Type:</b>	Full Length Article
<b>Full Title:</b>	Reinfection in leptospirosis: a 3-year retrospective study in two Pacific islands
<b>Abstract:</b>	<p>Background: Recurrent episodes of leptospirosis remain scarcely described. The objective of the study was to assess incidence and characteristics of reinfection by leptospirosis in a large at-risk population of the Pacific region.</p> <p>Methods: We performed a longitudinal study to capture patients admitted for leptospirosis more than once over a 3-year period in New Caledonia and Futuna Island (2007-2009).</p> <p>Results: Among 446 individuals admitted for confirmed leptospirosis, 5 cases of reinfection were identified in New Caledonia (2 cases) and Futuna (3 cases) during the study period. All recurrent episodes occurred in adults who presented with fever (100%), jaundice (80%), conjunctival suffusion (80%), headache (80%), and myalgia (80%). Renal function impairment was noted in 2 cases. No Weil disease nor fatality cases were observed. The mean time elapsed between the two episodes was 23 months. The serogroup was identified in all recurrent cases and was similar to the previous episode in 2 (40%) cases.</p> <p>Conclusions: Recurrent episodes of symptomatic leptospirosis appear to be non-severe, possibly due to awareness of the medical community. The observed low incidence of symptomatic reinfection may be explained by the potential role played by individual immunological factors in the physiopathology of the disease.</p>
<b>Manuscript Number:</b>	TRSTMH-D-16-00219
<b>Order of Authors:</b>	Gilles Guerrier Chantal Chouvin Eric D'Ortenzio
<b>Suggested Reviewers:</b>	
<b>Opposed Reviewers:</b>	

**Studies that reported information on age and gender proportions of cases and deaths from leptospirosis.** This table includes studies with information on cases or deaths according to gender and the following age-groups: 0-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70+ years.

Reference no.	Location	Cases	Deaths
[1]	Mayotte	63	1
[2]*	Réunion	199	16
[3]	Seychelles	75	–
[4]	Australia	825	–
[5]	Barbados	215	–
[6]	Ireland	116	–
[7]*	Netherlands	1037	34
[8]*	Salvador, Brazil	1438	105
[9]	New Caledonia	1811	7
[10]	Wallis and Futuna	232	1

–, No data reported; \*indicates the three studies that met criteria for calculating age and gender specific risk for death from leptospirosis.

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