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Human Leptospirosis in New Caledonia: Epidemiology, Laboratory Diagnostic Changes and Microbiology Patterns from 2006 to 2016, and Comparison with Previous and Regional Findings

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Abstract

With over one million cases worldwide yearly and high fatality in symptomatic forms, human leptospirosis is a growing public health concern for the most vulnerable populations, especially in the global warming and uncontrolled urbanization context. Although the Asia-Pacific region is particularly affected, accurate epidemiological data are lacking.

We conducted an eleven-year retrospective laboratory-based epidemiological survey in New Caledonia and compared our results with previous and regional data. From 2006 until 2016, we identified 904 cases, affecting mostly young males. We observed a clear seasonality with an annual peak in March-April. Our results demonstrate that leptospirosis remains highly endemic in New Caledonia with an average annual incidence of 30.6/100 000 and a case fatality rate of 3.2%. Over the period, there was a major shift from indirect serological diagnosis by MAT to direct diagnosis by real-time PCR, a more specific and sensitive test when performed early in the course of the disease. The systematic implementation of genotyping informed on the variety of the infective strains involved, with a predominance of serogroups Icterohaemorrhagiae and Pyrogenes. This time series showcases the trends of leptospirosis biological diagnosis to modern molecular approaches with an impact on epidemiological surveillance.

Keywords: Leptospirosis; Weil disease; New caledonia; Pacific islands; Polymerase chain reaction; Melanesia

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Introduction

Leptospirosis is among the most common zoonoses worldwide and an increasing public health concern [1,2]. Humans are accidentally infected by pathogenic *Leptospira* species principally from contaminated soils or water, or direct contact with rodents or other reservoir mammals in rural areas or flood-prone urban slums. In the tropics, leptospirosis remains a disease of poverty [3,4].

Following an incubation period of 2 to 26 days (average 10 days), symptomatic leptospirosis generally presents as a fever of abrupt onset, rigors, myalgia and headache, and an estimated 10% to 20% develop severe disease with approximately 10% fatalities [5]. Despite the efficacy of largely available antibiotics when provided early enough, the mortality of severe presentations and global public health costs of leptospirosis remain high [6]. Supportive care with renal replacement therapy, artificial ventilation and blood products needed to support patients during critical complications are lacking in most affected countries [7].

The World Health Organization's (WHO's) Leptospirosis Burden Epidemiology Reference Group estimated that 1.03 million cases occur worldwide annually with 58 900 deaths, the incidence in the tropics being approximately 10 times higher than in temperate regions [5]. Areas with the highest morbidity and mortality are located in the Asia-Pacific Region, the Pacific Island Countries and Territories (PICTs) facing the highest burden with a peak of 150 cases/100,000 population

Table 1: Number and estimated annual incidence of laboratory-diagnosed leptospirosis cases by age group and sex in New Caledonia from 2006 until 2016.

Age group (years)	N diagnosed cases			Population (2014)			Estimated annual incidence of reported cases in NC p. 100,000 pop.		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
[0-9]	28	13	41	21 441	20 231	41 672	11.9	5.8	8.9
[10-19]	129	47	176	22 574	21 742	44 316	52.0	19.7	36.1
[20-29]	117	47	164	20 509	19 790	40 299	51.9	21.6	37.0
[30-39]	120	62	182	20 098	20 295	40 393	54.3	27.8	41.0
[40-49]	91	52	143	19 969	19 784	39 753	41.4	23.9	32.7
[50-59]	52	41	93	14 566	14 239	28 805	32.5	26.2	29.4
[60-69]	39	27	66	9 752	9 217	18 969	36.4	26.6	31.6
≥ 70	28	11	39	6 633	7 927	14 560	38.4	12.6	24.4
total	604	300	904	135 542	13 3225	268 767	40.5	20.5	30.6

per year in Oceania [5,8]. Effective surveillance systems with appropriate laboratory support are urgently needed, especially in the disease-endemic, developing countries of this region [9,10]. Where implemented, the epidemiological surveillance of leptospirosis is classically based on serological data, using the Microscopic Agglutination Test (MAT), which is poorly sensitive in the first days following disease onset, highlighting the benefits of a combination of techniques including modern molecular diagnostic tests [11].

New Caledonia (NC) is a French territory currently governed under the Nouméa Accord, located in the southwest Pacific Ocean, about 1,210 km east of Australia. Its climate is subtropical: rainstorms predominate on the east coast, December to March being particularly rainy. Its population was 268 767 inhabitants in the 2014 census, 74.4% of whom lived in the South Province where the capital Nouméa is situated [12]. The 39% of the population who declared being part of the Melanesian Kanak community are located mostly outside Nouméa, frequently in rural tribes.

The first reports of human leptospirosis in NC date back to 1954, and it was thought to have been imported with boat rats after the start of colonization in 1853. The Institute Pasteur of New Caledonia (IPNC) studies this disease since the 1980s and has developed diagnostics for humans and animals [13,14]. Diagnosis was conducted exclusively at IPNC before being transferred to the Territorial Hospital in late 2016. We aimed to review the epidemiology of leptospirosis cases in NC in order to better inform local and regional Public Health interventions.

Methods

We reviewed laboratory-diagnosed human leptospirosis cases in NC from 2006 to 2016 and compared our results with previous data. Data for the period 2006 to October 2016 originated from the Institute Pasteur of New Caledonia (IPNC) laboratory database and for November-December 2016 from the Territorial Hospital Laboratory of New Caledonia (CHT). Samples originated from all medical structures: Territorial and Provincial Hospitals; Peripheral Health Centers; Private Clinics; General Practitioners and Private Laboratories.

All leptospirosis cases and associated anonymized demographic data were included in the study. Both early molecular diagnosis using a real-time PCR from serum or urine and late serological diagnosis using MAT with a panel of *Leptospira* strains of epidemiological relevance were used [15,16].

Leptospirosis cases were classified as confirmed (a positive qPCR or seroconversion in paired samples) or probable (having both a clinical presentation compatible with leptospirosis and a single MAT titer ≥ 800). The putative infecting serogroup was defined as the pathogenic serogroup yielding the highest titer in MAT serology. In addition, genotyping using a diagnostic PCR provided a putative serogroup for qPCR-confirmed cases [11].

Annualized incidence data (cases over 100,000 person-year) were compiled and graphed using Excel (Microsoft Corporation, Redmond, WA., USA). Because leptospirosis is a notifiable disease since 1991 in NC, patients were informed that their anonymized data could be used for surveillance and reporting purposes. No further ethical approval was therefore required.

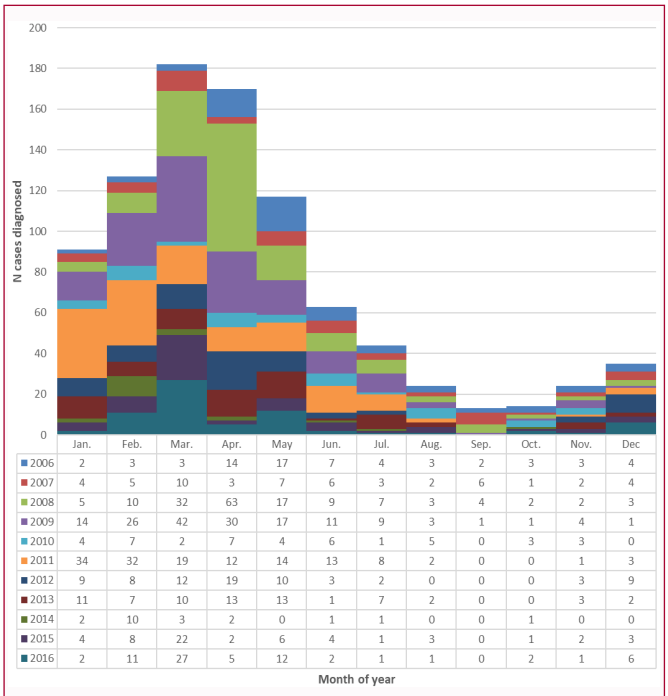
Results

Between January 1st, 2006 and December 31st, 2016, 904 human leptospirosis cases were diagnosed in NC. Of these, 204 were classified as "probable" and 700 as "confirmed".

The average annual lethality was 3.2% (range: 1.8% to 5.7%). The sex ratio was unbalanced with 604 males/300 females. The mean age was 35.6 years (median: 33.6; range 1.1-84.8 years). The most affected population subgroup was males aged 10 to 39 years with a mean annual incidence above 50/100,000 population (Table 1).

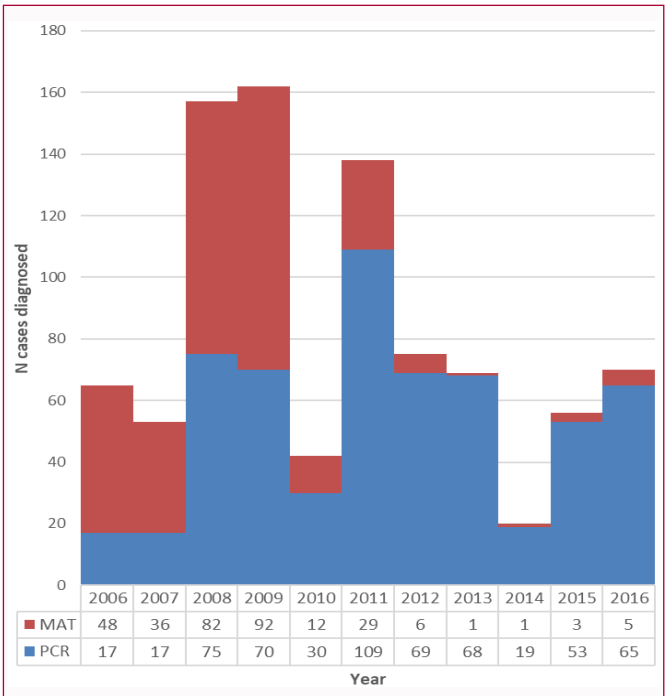
The mean annualized incidence rate of laboratory-diagnosed leptospirosis during the period was 30.6/100 000 population per year, ranging from 7.44 in 2014 to 59.16 in 2009. Our results show no global increase in the annualized incidence during the study period. The highest incidence rates were found among persons residing on the North-East Coast and in the suburb of Nouméa. Outbreaks occurred in 2008, 2009 and 2011. The cumulative number of human leptospirosis cases per month over the 11-year period is shown in Figure 1.

We observed a shift in biological diagnosis between 2006 and 2016: MAT serology was the confirmation method most used during the first years and was progressively replaced by Polymerase Chain Reaction (PCR) from 2010 onward (Figure 2). The 2006-2009 period was associated with <50% diagnosis using qPCR, while that method ensured >70% of diagnoses from 2010 onward and >90% since 2012. Increased use of qPCR was associated with a statistically significant increase in the percentage of confirmed diagnoses: during these two periods, the percentage of confirmed cases rose from 167/434 (61.5%) to 433/470 (92.1%) (Fisher p-value <0.001), likely due to the difficulty to access a convalescent serum sample from probable cases (Figure



	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec
2006	2	3	3	14	17	7	4	3	2	3	3	4
2007	4	5	10	3	7	6	3	2	6	1	2	4
2008	5	10	32	63	17	9	7	3	4	2	2	3
2009	14	26	42	30	17	11	9	3	1	1	4	1
2010	4	7	2	7	4	6	1	5	0	3	3	0
2011	34	32	19	12	14	13	8	2	0	0	1	3
2012	9	8	12	19	10	3	2	0	0	0	3	9
2013	11	7	10	13	13	1	7	2	0	0	3	2
2014	2	10	3	2	0	1	1	0	0	1	0	0
2015	4	8	22	2	6	4	1	3	0	1	2	3
2016	2	11	27	5	12	2	1	1	0	2	1	6

Figure 1: Cumulated monthly cases of human leptospirosis in New Caledonia during 2006 to 2016.



	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
MAT	48	36	82	92	12	29	6	1	1	3	5
PCR	17	17	75	70	30	109	69	68	19	53	65

Figure 2: Laboratory diagnostic methods used for human leptospirosis diagnosis in New Caledonia from 2006 to 2016.

2). We examined the association between qPCR use and deaths using lethality data from DASS-NC: lethality was 12/437 (2.7%) during 2006-2009 and 17/475 (3.6%) during 2010-2016, respectively (Fisher test p=0.57).

The infecting strain was putatively identified for 743 cases, by MAT serology (385 cases) and genotyping (449 cases). In all but 5 cases with both results available, there was an agreement between serology and genotyping. Disagreements between techniques were all observed in early serum samples with low MAT titers. In these 5 cases, the genotype was used to infer an infective strain. In total, the serogroup involved were Icterohaemorrhagiae (426 cases, 57.3% of informed cases), Pyrogenes (148 cases, 19.9%), Australis (86 cases, 11.6%), Ballum (47 cases, 6.3%) and Pomona (12 cases, 1.6%). In addition, Panama (13 MAT results), Canicola (n=8), Autumnalis (n=1) and Tarassovi (n=1) were suggested by MAT results but never evidenced by genotyping and were also never isolated in culture [11,17]. Of note, an infection by *Leptospira weilii* was known to have been acquired overseas [15]. We observed a change in this pattern over the eleven-year period with an increase in Icterohaemorrhagiae and a decrease in Canicola serogroups. We believe these results are probably not explained by the epidemiologic trends (geographical and animal origin of species) but could be caused by laboratory artifacts due to changes in the diagnostic technique. To the best of our knowledge and based on isolates [11], Panama and Canicola do not circulate in NC but are identified by poorly specific MAT tests because these are very reactive, leading to cross-reactions [17,18].

Discussion

While many observational leptospirosis studies were conducted in French Polynesia [19-21], other specialized laboratories are only available in Hawaii and New Caledonia to provide accurate data on this zoonosis among PICTs [22,23]. We explored the trends in New Caledonia (NC) as leptospirosis is feared to be an emerging disease due to global warming favoring floods, the poor housing in some settings increasing rat-human contacts [24].

Our study shows that leptospirosis has circulated intensely in NC over the period 2006-2016 inclusive, with a mean annualized incidence of 30.6/100 000 per year. As a comparison, 57 cases of human leptospirosis were reported in the population of 145,308 in NC between June 1983 and May 1985 (annualized incidence 19.6/100 000), and 192 during the 1989-93 period in a 164,173 population (annualized incidence 23.4/100 000). Lastly, with 239 cases and an estimated population of 225,296 the annualized incidence was 21/100 000 in the 2001-2005 period [13,16,25].

Comparing our findings with the other French Territories using similar diagnostics, NC incidence was higher than in Guadeloupe (where it declined from 25 to 6.9 annual cases per 100,000 population in 2016), Martinique (14.2 to 6.1), Mayotte (13.1), La Réunion (11.3), or French Guyana (5.9), but lower than French Polynesia (46) or Futuna (844, the highest incidence in the world) [21,26-28]. The incidence reported in NC is almost 100 times higher than in Mainland France for the study period [28]. In comparison to regional data, NC leptospirosis incidence is particularly high in intertropical zones of South-East Asia, in the Pacific Region, and in Australia where it is declining [9,13,19,26,29,30].

We observed a relatively low overall case fatality rate of 3.9% from 2006 until 2016, while it was 5.4% in the 2001 to 2005 period and 9.9% in 1989 to 1993 [13-16]. This slow but steady decrease in lethality (Chi-square for trend at 0.0002 and Chi-square for linearity <0.001) is in favor of improved global care in New Caledonia. Lethality in NC is now similar to that in Guadeloupe, Martinique and La Réunion [27,28].

Our demographic findings show that men aged 10 to 40 are overrepresented among diagnosed cases, with a sex ratio of 2.01 M/F. In a 1982 publication, the link between leptospirosis and being of Melanesian or Polynesian ancestry had been suspected, as were poor

hygiene, bathing in rivers, some occupational activities and living in suburban shantytowns of Nouméa [31]. Exposure was investigated in a 2002 to 2009 study, evidencing animal exposure (rodents, horses, bovines and pigs) as well as recreative activities (river fishing and bathing, hunting) as associated factors [23]. In 135 confirmed cases in 2008, contact with animals (OR>2), swimming, fishing and hunting (OR>3) were associated with the occurrence of cases [32,33]. Clinical studies including case investigations through questionnaires between 2006 and 2010 reported as important factors for human leptospirosis and common between the studies: contact with rats or domestic animals, walking barefoot in water or mud, swimming in rivers, gardening, farming and working in piggeries. Recent findings suggest that although major animal reservoirs of human-infecting leptospirosis may vary across the PICTs, livestock (especially cattle and pigs), dogs and rodents may all play important roles in disease transmission to humans [26,33]. In Australia and New Zealand occupational exposure has been described among cattle raising and slaughtering workers, livestock farmers and forestry-related workers [34,35].

Our compiled data over the 11-year period confirm a strong seasonality with an annual peak during the March-April hot and rainy season and after the December-February Austral summer school holidays in NC. In 1982 the link between rainy periods and leptospirosis in NC had already been suspected [36]. In the 1990s leptospirosis was declared endemic in NC with epidemics occurring during hot rainy periods [13]. The importance of the El Niño Southern Oscillation (ENSO), the main regional climatic phenomenon, has then been suspected [16]. El Ninõphases of the ENSO are associated with dry weather in the West Pacific including NC and fewer leptospirosis cases [32]. In 2008, 2009 and 2011, heavy rainfalls associated with floods were associated with the occurrence of outbreaks [32,33]. Regional publications show that leptospirosis has been associated with floods in Australia and Hawaii as well [37-39].

The genus *Leptospira* now contains 64 species of which 17 are recognized or putative pathogens, 21 are of intermediate or unclear pathogenicity, now classified in the "Pathogen-2" subclade and 25 are nonpathogenic saprophytic species that do not infect animal hosts [40]. The *Leptospira* species identified in human disease in NC are only *Leptospira interrogans* and *Leptospira borgpetersenii* [11,17]. The serogroups involved in human cases in our study period were predominantly Icterohaemorrhagiae, Pyrogenes and Australis, with additional cases caused by Ballum and Pomona serogroups. In the 37 cases from 1973 until 1980, serogroup Icterohaemorrhagiae was quasi exclusive [41]. The results from the 2001 to 2005 period, on 239 biologically confirmed leptospirosis cases serogroups were also mainly Icterohaemorrhagiae (69%), Australis (8%) and Pyrogenes (6%) [16]. In the 2008-11 retrospective case-control study of hospitalized patients in the Territorial Hospital, one of the risk factors for the development of severe leptospirosis was to be infected by *Leptospira interrogans* serogroup Icterohaemorrhagiae [6].

The diagnostic pattern changed dramatically over the eleven-year period of our study due to the large-scale use of qPCR test since 2010. qPCR is highly specific and can be used to diagnose leptospirosis in the first few days of the disease in a definitive way, while serological confirmation requires repeat testing which is often challenging due to patient loss to follow-up. During the 2008 outbreak, early diagnosis using qPCR allowed the diagnosis of 54% of the NC cases [33], a proportion that has reached more than 95% in the last years of our study period. The increase in qPCR-based diagnoses from 2006 to 2016 could optimistically suggest earlier patients' consultation or clinicians' testing with qPCR in the blood or in the urine that could contribute to reduce the risk of complications through the prescription of an antibiotic therapy [6,42]. We observed a decrease in lethality over the past three decades which was divided by two, probably thanks to earlier diagnosis, improvement of patients' transportations and medical care. However, we found no statistical association between lethality and greater PCR use in NC. In the French West Indies, inclusion of PCR and IgM ELISA tests for diagnosis of leptospirosis resulted in an increased sensitivity in comparison with MAT [27]. However, serology lacks specificity and remains positive long after the frequent self-limiting *Leptospira* infections, the level of the antibody titers cannot be used to predict the infecting serogroup, and false-negative results may occur if infection involves a serogroup not included in the MAT panel at a given reference center [43]. Hopefully, more accurate rapid diagnostic tests will be validated and become widely available in the near future [44]. Such rapid tests are needed for leptospirosis, especially in areas in which arboviruses, hemorrhagic fevers and malaria are common differential diagnoses. Our study may suffer from biases and limitations. First, changes in diagnostic methods during the study period may have led to a bias in the number of diagnosed cases. MAT use could have somewhat overestimated the number of cases in an endemic territory like NC where repeated infections may cause antibodies to rise above detectable levels in a prolonged manner, and MAT positivity in absence of ongoing infection [45]. However, we used a ≥ 800 titer to define a probable case, a very high threshold that is most likely to reflect recent infection. Therefore, changes in diagnostic methods is unlikely to have a significant impact on our data. Second, the potential changes in serogroups we observed may not be an accurate reflection of a real change of the epidemiology of leptospirosis in NC. As discussed above for the apparent decrease of serogroups Canicola and Panama, evidences suggest that genotyping provides better insight and that apparent changes in circulating strains are likely due to artefactual identification of cross-reacting serogroups through MAT. Third, only patients developing severe disease are likely to be tested, leading to surveillance bias and underestimation of the true incidence. This is especially the case in rural areas of NC, where patients may not even seek medical advice and clinical practitioners often do not resort to diagnosis in ambulatory patients and directly administer probabilistic antibiotic treatment. Although leptospirosis can be fatal, infection is often pauci-symptomatic or benign [46,47]. As in most other diseases, our data therefore mainly pertain to the progression of comparatively severe leptospirosis cases which came to the attention of the health care system. These, however, remain a significant public health concern in NC. Fourth, we observed no significant reduction in lethality in NC during 2006 to 2016 despite the increased use of qPCR after 2009. This may be due to small numbers of nevertheless diverse patients, leading to the difficulty to detect even strong associations. Another explanation could be that patients in the 2010 to 2016 period suffered from more comorbidities than patients during the 2006 to 2009 period, these factors having been shown to be associated with poorer outcome in untreated leptospirosis. The median age of patients, however, was comparable for the two periods, at 34.3 and 32.9 years, respectively. Finally, switching from MAT to PCR may have accelerated diagnostic confirmation but time to first antibiotic treatment may have remained the same due to clinicians in NC being more aware of leptospirosis, prescribing syndromic antibiotic treatment well before obtaining diagnostic confirmation.

As opposed to earlier antibiotic treatment, there is little evidence that earlier diagnosis improves patient outcome, however it simplifies clinicians' task, especially in a subtropical area with several differential diagnoses and may provide more reliable data.

Conclusion

We report an updated epidemiological description of diagnosed human leptospirosis cases in New Caledonia over a period of 11 years and analyzed laboratory diagnostic changes, compared with the previous and regional available data.

Our study shows clear seasonality: health professionals and facilities should be prepared to test and treat more leptospirosis patients from February until May each year in NC. Young men still pay the biggest tribute to leptospirosis and should be the target of information and prevention campaigns. Improved and better targeted prevention messages are needed to avert human leptospirosis, avoidable deaths and related healthcare costs.

Like in the French West Indies and New Caledonia, molecular diagnosis (qPCR) should be made readily available to all humid tropical and subtropical resource-limited countries, including PICTs, who pay a high toll to this neglected disease. This should improve ease of diagnosis and provide accurate, comparable and reproducible epidemiological surveillance data to inform local authorities.

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