**Leptospirosis as a major public health concern in New Caledonia: the need for a multidisciplinary approach**

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**ABSTRACT**

In this paper, we describe the multidisciplinary approach that we are setting up to study leptospirosis and its complex eco-epidemiology in New Caledonia. Based on an assessment of the major research needs for this neglected disease both at the global (world) and local (New Caledonia) levels, we structured our research program with three workpackages that are presented and discussed.

**Keywords**
Leptospirosis, New Caledonia, epidemiology, reservoirs, diagnosis, prognosis.

1. **INTRODUCTION**

Leptospirosis is a zoonosis of worldwide distribution with major incidence in the tropics [1]. In New Caledonia, leptospirosis has been studied for years and is regarded as a major public health concern. It is characterized by an endemic pattern with seasonal epidemics arising during rainy periods, notably during “la Niña” periods [2-5]. Both the endemicity and epidemics have a classical rural distribution and result from the infection by a variety of serovars [2].

The Institute Pasteur in New Caledonia (IPNC) is the local reference laboratory for diagnosing human leptospirosis and has been studying this disease for years, notably developing and implementing efficient molecular diagnostic tools. At the same time, an expertise on this disease has led to numerous studies on epidemiology and virulence mechanisms of pathogenic leptospires [6-11]. More recently, studies were developed aiming at the understanding of the contribution of the host immune response to the clinical expression in severe forms of leptospirosis[12].

Because leptospirosis is a complex pathology involving reservoir hosts, environmental sources of infection, susceptible mammals including Man and the expression of the disease also depends on particular host factors [1,13], we have launched a multidisciplinary research program on this disease.

2. **ASSESSING MAJOR RESEARCH NEEDS**

Leptospirosis is recognized a “Tropical Neglected Disease” [14,15] and research needs have been discussed and highlighted [16]. These needs and priorities were evaluated in the new Caledonian context and from a world-wide point of view.

In any region, a limited number of pathogenic *Leptospira* strains are circulating and can cause disease in humans. Every single strain is supposed to be maintained by one or several reservoir animal host species. Particular exposition situations are then associated with environmental exposure to these pathogenic leptospires. Because the control of human leptospirosis has to include these animal and environmental factors, a study aimed at deciphering the eco-epidemiology of leptospirosis in New Caledonia has been started.

One major weakness highlighted by World Health Organization [14,15] is the diagnosis, which is highly specialized and time-consuming (serology) or requires highly specialized technologies (molecular diagnosis). We therefore decided, as one of our workpackages to develop and validate a Rapid Diagnostic Test (RDT) that could notably be used for early diagnosis.

Another difficulty for clinicians is to forecast the progress of a clinical leptospirosis. Whether it will tend to be a self-limiting disease with a rapidly favorable outcome or to a severe form with multiple organ failure is often hard to evaluate. Because this prognosis is necessary to decide on a hospitalization, another workpackage is aimed at finding prognosis indicators of outcome in clinical leptospirosis.

3. **DECIPHERING A COMPLEX ECO-EPIDEMIOLOGY**

At a global level, the epidemiology of leptospirosis is known from early studies. The focal point is animal reservoir populations, in which carrier individuals shed pathogenic leptospires in their urine. Humans become infected either from the direct contact with the urine or infected organs of the reservoir hosts (direct exposure) during particular occupations (vets, butchers, hunters, pet-owners …) or via the contact with environments that have formerly been contaminated with the urine of reservoir animals (indirect exposure). The indirect exposure, which is considered as the main contamination mode, is also associated with particular
professional (sewage workers, soldiers on maneuver...) or leisure (freshwater swimming or other outdoor water sports) occupations.

Beside this global scheme, early studies have also allowed to determine the associations that exist between particular infecting serovars and their reservoir animals. Based on these known associations, particular preventive measures can be proposed to targeted populations based on an assessment of the exposure risk. However, on a regional basis, the knowledge of these suspected serovar - reservoir associations has only limited implications. Because the studies have been conducted in different locations, they included possibly different animal reservoirs and different circulating Leptospira strains. Additionally, because the genetic basis of the serological taxonomy of leptospires was shown not to be monophyletic [17,18], strains considered as belonging to the same serovar from different locations can have strictly different animal reservoirs and different epidemiology.

Therefore, precisely identifying strains infecting humans and their animal reservoirs in regional studies may allow a better understanding of human exposures and thereby to better target prevention and control of leptospirosis.

The ecology of leptospires outside of their Mammal hosts has been studied with serological and culture tools in early studies [19-21]. However, these studies did not always separate pathogenic and saprophytic leptospires. Therefore, the ecology of pathogenic leptospires outside Mammal hosts remains very poorly documented.

We therefore designed a study aimed at deciphering the eco-epidemiology of leptospirosis in the new caledonian context, an archipelago with a limited number of Mammal species and a high incidence of human leptospirosis, resulting from both a known endemicity and seasonal epidemics. In both endemic and epidemic cases, human infections are believed to result either from direct (animal) or indirect (environmental) exposures, the latter probably being largely predominant.

3.1 Choosing a study site

Leptospirosis is known to have a rural epidemiological pattern in New Caledonia and regions with highest incidences have been identified [2,3]. During the first semester of 2008, an epidemic of leptospirosis occurred under the influence of heavy rainfalls causing a number of floods. We used the data from this epidemic to better specify the hot spots of highest incidence. Three districts in the Bourail region were identified as having the highest incidence (figure 1). Two of these were chosen for a field study of the eco-epidemiology of leptospirosis, based on our ability to conduct the study with the populations involved.

These two study sites correspond to two Melanesian tribes where many outdoor activities are part of the everyday life, including fishing and bathing in freshwater streams, agriculture, backyard pig pens, hunting (deer and wild hogs). Most of the households have one or more dogs that freely stray from the houses. Many of the inhabitants frequently go bare foot and know the presence of rodents in and around the houses.

Fresh water is supplied in every household by a council supply system but the use of the freshwater streams that crosses the tribe is very frequent.

3.2 Animal samples

Sera and urines from domestic animals (cattle, dogs and horses) are obtained from the veterinarian surgeon of the council, based on clinical suspicion of leptospirosis (we are very grateful to Dr S. Birna-Blum, DVM, for this useful contribution). They are given free of charge provided the diagnosis results are also returned free of charge.

Additionally, blood and kidney samples will be collected at the slaughterhouse from animals (cattle and deer) reared in the vicinity of the study site when available. Lastly, hunters were requested to provide deer and wild hog kidney samples when possible but none of these samples have yet been obtained.

3.3 Rodents samples

No native terrestrial Mammal is known from New Caledonia, except bats (both micro- and megabats) [22]. Four rodent species are known to be present in New Caledonia, all resulting from importation by the early human settlements: three rat species (Rattus rattus, Rattus norvegicus and Rattus exulans) and the domestic mouse (Mus musculus) [23].

Rodent populations are studied in our study sites both in the hot rainy season (February - March) and during the cooler months (August - September). The sampling strategy is aimed at evaluating both the rodent abundance and the prevalence of Leptospira in the kidney of the different rodent species.

The method used is based on the standard technique developed in New Zealand for the study of rodents [24] : Rodent abundance is measured by trapping index. This method gives information on both the identification [24] and abundance of rodents [25]. It allows for the calculation of an index of abundance, which is used to compare populations in different regions, habitats or seasons. 100 snap traps are placed in pairs along a line transect close to the households of known leptospirosis cases (= 50 trap stations with one each of rat and mouse trap size). Trap stations are set for 3 consecutive nights and baited with peanut butter and cheese.

3.4 Human samples

Human samples originate from the leptospirosis diagnosis requests, the Institut Pasteur being the reference diagnosis laboratory for leptospirosis. Because PCR analysis of serum or urine samples accounts for 50% of the diagnosis, these PCR
positive samples provide information on the genotypes of the infecting strains (Guarant et al., submitted).

3.5 Environmental samples
Samples of water, soil and mud are collected along the line transect used for the rodent study, in sterile polypropylene containers. Surface soil samples are collected directly with the container that is immediately closed. Water is collected in the containers at a 10 cm depth when possible. At the time and on the site of sampling, the pH is measured using a field WTW pH-meter with a plastic electrode. Samples are stored at 4°C until being either handled within 24 hours of collection (water) or stored at -20°C (soils).

3.6 Sample handling and treatment
Human samples are handled as usual for the routine diagnosis as described before [26]. For animal sera, they are handled similarly except that the MAT positive threshold varies according to the animal species (titers of 50 for dogs, 100 for cattle and deer, 200 for horses). For kidney samples, one small piece of kidney is aseptically put into a EMJH culture tube supplemented with 300 µg.ml⁻¹ 5-Fluoro-Uracile as an inhibitor of contaminant bacteria [27]. The remaining kidney is individually identified and stored in 95% ethanol for postponed DNA extraction.

Water samples are handled and extracted within 24 hours of collection. All DNA extracts are stored at -20°C.

3.7 Molecular tools
DNA samples from environmental samples are studied with quantitative real time PCR on a LightCycler 2.0 platform using the lfb1 PCR [10] that allows the quantification of all pathogenic leptospires. Samples from humans and animals are studied with the same PCR and an additional PCR test conducted on a LightCycler 480 using Leptospira specific primers that amplify both saprophytic and pathogenic leptospires [6]. Additional molecular typing tools are also currently being developed.

3.8 Expected results
This study will allow identifying the Leptospira strains circulating and their animal reservoirs in the New Caledonian context. Data from the environmental samples should also allow identifying specific environments with high risk of leptospirosis contamination.

Taken together, this study will give a better knowledge of Leptospira ecology and epidemiology, providing the basis for the design of efficient control measures.

4. TOWARDS A RAPID TOOL FOR EARLY DIAGNOSTIC
The International Leptospirosis Society together with the World Health Organization point to the weakness of current diagnosis methods for leptospirosis. Actually, leptospires are slow-growing delicate bacteria that have specific nutritional requirements and the culture and isolation time is much too long for a clinically useful diagnosis. Other early diagnosis techniques use nucleic acids amplification techniques to evidence pathogenic leptospires in biological fluids (blood or urine), but these techniques are still too specialized to be used in peripheral health centers. Lastly, the serological reference technique uses the micro-agglutination test that requires the use of a panel of live cultures of leptospires, that can neither be set up in health centers.

Yet, a rapid diagnosis is a key factor of the outcome in leptospirosis [28]. The advent of RDTs based on immunochromatography has proved very useful in the diagnosis of a number of tropical diseases, especially in basic health centers settings [29,30].

Recent works on leptospirosis have highlighted a number of possible leptospiral targets that could be used for specific detection of Leptospira spp. in biological fluids [31-33].

Our current work is aimed at producing and evaluating various monoclonal antibodies (mAbs) targeting antigenic targets that are conserved among virulent Leptospira. These mAbs will first be tested with cultures of Leptospira reference strains at various dilutions then with known positive archive sera. They will then be used to design RDTs that will be further evaluated using archived Leptospira isolates and patient sera. These tests will then be implemented in peripheral health centers in New Caledonia. They will later also be tested for a veterinary use.

5. THE HOST RESPONSE AS AN INDICATOR FOR PROGNOSIS
Leptospirosis has a highly variable clinical figure. It ranges from asymptomatic forms to severe, often fatal, multiple organ failure. When facing a leptospirosis suspicion with a classical influenza-like syndrome, the clinicians have very little indications on the possible evolution.

The immune response to Leptospira was long believed to be strictly humoral, but recent works have highlighted both an in vitro [34,35] and in vivo Th1 response [12]. Whether this inflammatory response possibly contributes to severe forms of leptospirosis remains to be studied. Our hypothesis is that the intensity of this Th1 response might be a predictor of clinical evolution and disease outcome in patients with leptospirosis.

Using a hamster model of severe leptospirosis, we studied the immune gene expression patterns in individual animals and compared cytokine gene expression pattern in spontaneously recovering animals to the one observed in hamsters with a severe disease with fatal outcome. Our results (Vernel-Pauillac and Guarant, in prep) demonstrate that the gene expression level of some cytokines can actually be a good predictor of disease outcome in this animal model of leptospirosis.

We recently started a clinical study aiming at evaluating both the gene expression levels and serum concentrations of various cytokines in patients with acute leptospirosis. This study, conducted together with clinicians, could confirm the prognosis value of some of these indicators and provide additional knowledge on the complex pathogenesis of leptospirosis.

6. CONCLUSIONS
Leptospirosis is a recognized “Tropical Neglected Disease” and research needs have been highlighted. It is also a disease of complex ecology and epidemiology, being both a zoonosis and an environment-transmitted disease. The research needs and priorities were evaluated in the new Caledonian context and our research program was drawn from this evaluation, keeping in mind a world-wide point of view. We therefore designed a
research program focusing on diagnosis, prognosis and prevention.

Making the diagnosis more accessible and easier appears as a priority for leptospirosis worldwide and a bed-side easy and rapid diagnostic tool would be very useful. Putting together the expertise from the National Reference Center, Technological Platform specialized in the development of monoclonal antibodies with local diagnostic labs should allow the development and validation of a rapid diagnostic test available for routine use in field diagnosis. This tool will allow both a better and earlier diagnosis, which is a key determinant of leptospirosis outcome and a better surveillance. Subsequent developments could include its validation and use for the diagnosis of veterinary leptospirosis cases.

Clinical leptospirosis can have either rapidly favorable or fatal outcomes. Whether a patient with leptospirosis should be sent back home with the adequate treatment, remain at the hospital under surveillance or even be transferred to an intensive care unit is frequently hard to decide for clinicians. Evidencing parameters that would have a good prognosis value would be very helpful as decision helping tools for clinicians. Putting together researchers and clinicians will allow a precise assessment of the outcome of leptospirosis cases as a comparison with the study of particular parameters of their immune response. Because the immune response to leptospirosis is an integral part of the disease [36,37], a better understanding of the host response will also increase our understanding of disease pathogenesis in severe forms of leptospirosis.

Lastly, understanding the ecology and epidemiology of this zoonotic and environmental disease in the new Caledonian context deserves a multi-factorial approach. This part of our program puts together microbiologists, laboratory diagnosticians, clinicians, veterinary diagnosticians and ecologists. Based on the precise knowledge of circulating Leptospira strains, their reservoir hosts and environment survival capacities will provide the tools for a better assessment of the contamination circumstances. It should allow setting up innovative preventive measures.

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8. REFERENCES


