A Global Research Agenda for Leptospirosis

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Abstract

Leptospirosis is a zoonotic spirochetal disease of global importance. This disease continues to have a major impact on people living in urban and rural areas of developing countries with inestimable morbidity and mortality. Funding for research and control efforts is currently haphazard, not organized and not effective for public health efforts, primarily because there are no concerted, ongoing international efforts to assess the impact of leptospirosis on human health. Major issues in the field need to be addressed to develop strategies of control, amelioration and treatment. These include the following: mechanisms of naturally acquired and vaccine-induced protective immunity against clinical leptospirosis; mechanisms of severe leptospirosis pathogenesis; standardized, precise and simplified taxonomy of Leptospira relevant to disease manifestations, transmission and control; effective adjunct treatments in addition to antimicrobials; and environmental assessment for risk of leptospirosis transmission and relevant mammalian reservoirs. Once effective ongoing, collaborative international efforts to assess the impact of leptospirosis on human and veterinary health are underway, appropriate mobilization of clinical and public health research funding will follow.

Keywords

Awareness has increased internationally over the past decade that leptospirosis is a major globally important public health threat, both in developing countries and industrialized countries.[1] The economic burden imposed by this disease is unknown, yet it is widely recognized that the incidence of leptospirosis is remarkably underestimated and the disease underdiagnosed in endemic regions. Leptospirosis is estimated to affect tens of millions of humans annually with case fatality rates ranging from 5 to 25%.[2] Case finding and reporting have been limited, nonsustained and biased. Domestic veterinary loss is substantial but the burden unknown. More precise and systematic assessment of the impact of leptospirosis on humans, as well as on animals of economic importance, is critical for assuring the proper international support and priority for research in this disease. The goal of this article is to emphasize major issues in leptospirosis research that need to be addressed so that priority areas can move forward with adequate support.

Currently available data suggest that leptospirosis is the most common zoonosis worldwide. Although the disease is endemic in rural areas around the world, transmission continues in developed countries as well, for example in the US (especially tropical Hawaii[3,4] but also inner cities),[5,6] Ireland[7] and Germany.[8] Annual incidence reports of leptospirosis vary from 0.53 per 100 000 population in tropical areas to 0.04 per 100 000 population in developed countries such as Germany.[2,8]

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The leptospirosis aphorism: wherever leptospirosis is looked for, it is found

Leptospirosis is underreported due to lack of clinical suspicion and barriers to diagnostic capacity. General physicians often lack familiarity with the broad clinical presentation of leptospirosis. Most commonly, human infection with pathogenic *Leptospira* results in asymptomatic seroconversion.[9] Less commonly, leptospiral infection of humans results in symptomatic illness. When symptomatic, leptospirosis most often manifests as an undifferentiated febrile illness. Less commonly – in perhaps fewer than 5–10% of infections – severe disease results, which is manifested by any combination of jaundice, renal failure, hemorrhage, refractory shock, and myocarditis.[1] Major syndromes such as leptospirosis pulmonary hemorrhagic disease may appear in the absence of jaundice in areas of high prevalence of the disease, such as the Andaman Islands (as discovered in the seminal and now classic descriptions of leptospirosis as the cause of Andaman Hemorrhagic Fever by the group of Sehgal),[10–12] rural Nicaragua[13] or the Peruvian Amazon.[14,15] In the case of the Nicaragua epidemic, diagnosis was delayed due to confusion with other potential causes of epidemic febrile illness such as dengue and other viral hemorrhagic fever syndromes.[16] In urban epidemics that have occurred in some places such as Salvador, Brazil,[17] Weil's disease as manifested by jaundice and renal failure has not necessarily been associated with pulmonary hemorrhage,[17] although more recently, for unexplained reasons, pulmonary hemorrhage has emerged as an important clinical syndrome (AI Ko, personal communication). Numerous serosurveys and hospital-based case finding studies have been carried out,[3,8,11,14,18–28] reinforcing time and again that leptospirosis transmission is incredibly common yet poorly recognized.

Environmental assessment to assess leptospirosis risk

A better understanding of the epidemiologic determinants of leptospirosis is needed to better determine appropriate public health approaches to ameliorate leptospirosis transmission. Animal reservoirs of leptospirosis may be domestic livestock, peridomestic rats and mice, companion animals particularly dogs and potentially wild animals such as bats,[29,30] marsupials and a large variety of rodents.[29]

The zoonotic sources of infection vary in different regions. Leptospirosis has traditionally been considered mainly a disease of farmers. In places where cattle and pigs are raised, these animals are often chronically colonized by pathogenic *Leptospira* with frequent transmission to humans. Over the past decade, there is increased recognition that additional risk groups include urban dwellers[5,17] and adventure travelers. The outbreak of leptospirosis associated with the EcoChallenge in Borneo was seen worldwide in athletes returning to their home countries.[31] However, of high public health importance, the urban environment is an important place where transmission of severe leptospirosis takes place. In such places, rats likely predominate as the zoonotic source of epidemic disease in urban environments such as Salvador, Brazil[17] or Baltimore[5] and Detroit[6,32] in the US. Dogs must be considered as important sources of urban transmission, which may be transmitted from vaccinated[33] or dogs wandering in the community. In some regions, canine leptospirosis is considered an emerging infectious disease of veterinary and potentially zoonotic importance. Dogs have increasingly been noted to be infected by serovars not included in the commercially available vaccines, particularly serovar Grippotyphosa; raccoons are thought to be the source of infection.[34]

In endemic areas of leptospirosis, factors such as lack of sanitary conditions, mud flooring, together with rainy seasons and flooding catastrophes contribute to periodic outbreaks.[35,36] Interestingly, in this setting, many exposed people have asymptomatic seroconversion and some also undiagnosed fever; a small but important minority may develop severe disease. In Hawaii, a tropical region of the US where leptospirosis is common and is a major public health

concern [Figure 1], the zoonotic sources of leptospirosis transmission have not been precisely identified. The hazard of fresh water exposure for leptospirosis causes substantial alarm among tourists and local inhabitants, but as yet, there is no way to assess the true risk of infection for people there. In developing countries where proper sanitation is not maintained such as in *favelas* in Brazil or market and living areas of urban slums in the Peruvian Amazon, surface waters must be considered as representing substantial risk of leptospirosis transmission.

Quantifying the presence and determining types of pathogenic *Leptospira* in environmental surface waters would be an important tool for guiding leptospirosis control efforts in endemic regions, such as an outbreak that occurred in 2004 in a new village, Los Delfines, in the Peruvian Amazon region of Iquitos [Figure 2]. Such an approach would also have economic implications. For example, Hawaiian tourism has been negatively affected by unremediated leptospiral contamination of environmental surface waters [Figure 1]. Outbreaks of leptospirosis among elite athletes has occurred due to contamination of fresh water competition sites.[31,37] Culture methods are insufficient to address this task since saprophytic leptospires and other microbes have confounded such attempts in the past. We have been using a pathogen-specific quantitative PCR-based assay combined with molecular taxonomy to determine the types and quantities of pathogenic *Leptospira* in urban and rural areas in the Peruvian Amazon. Preliminary results indicate that in this region, far higher concentrations of pathogenic *Leptospira*, including *Leptospira interrogans* are present in the urban slum areas compared to rural water sources, where *Leptospira inadai* and *Leptospira santarosai* predominate but in far lower concentrations (C Ganoza, D Collins-Richards, MA Matthias, JM Vinetz, manuscript submitted). This approach promises to make a major contribution to assessing human risk for acquiring leptospirosis in relationship to activities of daily living for residents of endemic areas.

What determines the severity of leptospiral infection? Host *Vs* infecting strain

Why do only some people develop any clinical manifestations of leptospirosis such as undifferentiated fever,[9] and of these, few (less than 5–10%) develop severe disease[13] despite being infected with highly pathogenic leptospires such as *L. interrogans* serovar Icterohaemorrhagiae?[3] The discrepancy between infection and symptomatic and severe disease remains a central mystery. Possible explanations (summarized in Table 1) include considerations of both the pathogen and the human host. It has long been thought that certain leptospiral serovars are particularly pathogenic. For example, serovars Copenhageni and Lai, whose whole genome sequences have been determined have been associated with severe human leptospirosis. These serovars do not cause disease in their natural mammalian reservoirs (*Rattus* spp. and *Apodemus* mice, respectively) but cause severe disease in experimental models of infection such as in hamsters or guinea pigs or in accidental hosts such as dogs. In fact most people infected with these serovars probably do not develop severe disease, possible no disease at all. Serosurveys in urban areas of Brazil where serovar Copenhageni predominates as a single clonal subpopulation[38] show that many people with anti-Copenhageni antibodies never had severe leptospirosis.[28] Conversely, a wide variety of *Leptospira* have been shown to cause severe disease, including the 'intermediate' leptospire, *Leptospira fainei* serovar Hurstbridge,[39] even though, again, most infections are probably inapparent.[40] Therefore, intrinsic virulence properties of pathogenic or intermediate *Leptospira* cannot be the sole explanation of severe human disease.

Alternatively, human host genetics are likely to determine clinical outcomes of leptospiral infection. One report suggests the association of a major histocompatibility complex allele, HLA-DQ6, with risk of infection due to ingestion of *Leptospira*-contaminated water in an outbreak of leptospirosis in the US.[41] However, no investigations have been reported of human genetic susceptibility to severe leptospirosis. A major contribution to understanding the role of human immune factors in intrinsic resistance to the effects of pathogenic

Leptospira would be to develop an international multi-center, prospective study of well defined cohorts to create a specimen bank consisting of isolates, serum, and patient DNA samples. The recent publication of two leptospiral whole genome sequences[42,43] and recent advances in systems biology would facilitate microarray and proteomic analysis of leptospiral[44–46] and human host determinants of disease susceptibility and clinical manifestations.

Another potential determinant of leptospirosis severity could include the dose of *Leptospira* inoculated during environmental exposure. Such an explanation can now be addressed with the quantitative molecular methods for assessing leptospiral contamination of environmental surface waters.

Acquired and innate immunity to *leptospira* in endemic regions

Humans living in leptospirosis-endemic regions are commonly exposed to *Leptospira* during their activities of daily living. Therefore, it is likely that some people living in leptospirosis-endemic regions develop naturally acquired protective immunity against severe disease. What are the mechanisms of such protective immunity in humans? Serovar-specific antibodies targeted against lipopolysaccharide or antibodies against cross-species conserved proteins such as LipL32 or others?[46] Does acquired cellular immunity with either T cells with ab T cell receptors or T cells with gd T cell receptors playing an important role.[47–49] Are naturally acquired protective immune mechanisms long- or short-lived since protective immunity induced by bacterins (killed whole cell vaccines) are short-lived? What *in vitro* tests or small animal models can be developed to assess naturally acquired protective immunity in humans? Do such responses affect disease pathogenesis? Can naturally acquired immunity be used to guide vaccine development for humans?

Some of the reasons may relate to transmission intensity and pre-existing population immunity, virulence of circulating leptospiral serovars and/or host immunogenetic factors. Data are limited regarding the exact contribution of each of these variables. It seems intuitive that pre-existing immunity due to previous exposure protects against subsequent infection. However, no published data either support or refute this concept. Of note, anti-leptospiral antibodies, as detected by the microscopic agglutination test, can be short-lived (in the order of months), or May last years. It is also possible that pre-existing immunity may influence the severity of a subsequent leptospiral infection. No evidence suggests that an immune sensitization phenomenon predisposes to severe leptospirosis as occurs in dengue virus infection, where a second infection with a different dengue virus serotype increases the risk for dengue haemorrhagic fever. Future prospective studies are needed to address the central issues of the role of acquired and innate immunity in protection from leptospirosis. Such studies would include establishing cohort studies in endemic areas in which risk for symptomatic leptospirosis during primary *Vs* subsequent exposure is quantified. Being able to precisely determine the infecting leptospiral strain would be essential to such studies. To determine whether human protective immunity develops in exposed population requires the development of experimental methods (*in vitro* or small animal) to determined correlates of acquired and innate protective immunity against leptospirosis in humans.

Identification of leptospiral isolates

A common problem in leptospirosis is how to identify infecting *Leptospira* isolated from clinical specimens, from animals, and from environmental sources. *Leptospira* are conventionally classified phenotypically by a cumbersome and outdated serologically based scheme with expensive polyclonal and monoclonal antibody reagents and reference strains available only from reference laboratories. The gold standard serological classification, the cross agglutination absorption test (CAAT) is labour-intensive, requires a large panel of live leptospires, takes months to complete and, being phenotypic, does not reflect genetic

relatedness. The most comprehensive classification scheme is based on DNA relatedness among members of the family *Leptospiraceae,*[50] a technique not available outside of reference centers. Many molecular methods have been applied to the characterization of leptospiral isolates, including restriction enzyme analysis, pulse field gel electrophoresis, random amplified polymorphic DNA analysis and arbitrarily primed PCR fingerprinting.[51,52] 16S ribosomal RNA gene sequence analysis can distinguish leptospiral species[53,54] but whether it has sufficient discriminatory power to distinguish among strains (i.e. serovars) within a species has not been shown.

A major priority of international collaborative research efforts should be to create a comprehensive *Leptospira* database curated by an expert group. This database should contain reference information for all known *Leptospira*. A comprehensive entry for a leptospiral isolate might include:date of isolation, source (human, animal or water source), clinical outcome of patient (if applicable), region, serological typing CAAT, monoclonal antibody, DNA-DNA hybridization, PFGE, RAPD, and AP-PCR patterns (under standardized conditions), 16S ribosomal RNA gene sequence. This database should be accessible using appropriate informatics tools through an Internet browser interface. The service should be provided gratis and be timely. The goal should be to provide a resource for the rapid identification of infecting leptospires for clinical, epidemiologic and basic research purposes.

Conclusion

Recent worldwide progress in leptospirosis compels increased international attention towards addressing its role in clinical and public health. Organizations such as the International Leptospirosis Society should be the focal point for developing international co-operative efforts. Agencies such as the World Health Organization, the Food and Agricultural Organization, the Pan American Health Organization, the US National Institutes of Health and the European Union should be approached for sustained support to address some of the following central problems of international importance:

- Development of an international registry of cases with an estimate of incidence and prevalence, to provide policy guidance to public health authorities.

- Creation of regional and national networks of leptospirosis diagnostic laboratories with appropriate quality assurance controls.

- The establishment of central repositories of leptospiral strains, hybridomas, reference polyclonal antisera and monoclonal antibodies, available at nominal cost to qualified laboratories.

- Creation of an international, internet-queriable comprehensive Leptospiral database.

- Development of an international, multi-center, prospective cohort study of the human, leptospiral virulence factors, and environmental determinants of leptospirosis clinical manifestations and clinical outcome. This would involve creation of a specimen bank from well-characterized leptospirosis patients and environmental and zoonotic sources to include: leptospiral isolates; clinical samples of blood and urine for molecular identification of leptospires and evaluation of new diagnostic tests; patient DNA samples for determining genetic associations with different outcomes of leptospiral infection; and environmental water samples from endemic and epidemic regions accompanied by GPS and geographic data to determine risk of transmission and infection.

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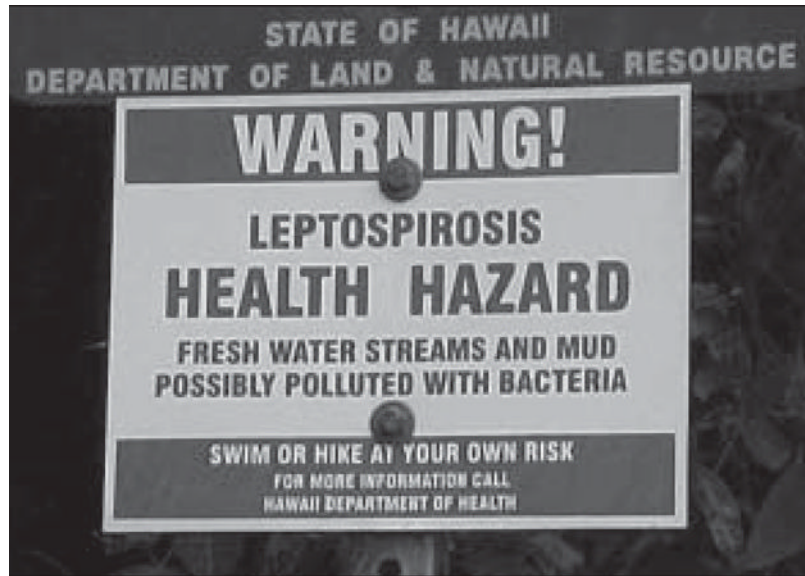
Figure 1.
Warning sign in Hawaii, US about the risk of environmental exposure to leptospirosis

Figure 2.
An outbreak of leptospirosis occurred in a swimming hole (left) at the edge of a newly developed village, Los Delfines, 10 km outside the city of Iquitos in the Peruvian Amazon. This outbreak resulted in the death of three children who had renal and respiratory failure, and cutaneous and gingival haemorrhage. Sixty-seven people sought medical attention for undiagnosed fever. Quantitative analysis of the creek running from the pig farm to the swimming hole showed that a new pig farm upstream of the village was the source of the outbreak. The Directorate of Public Health (DISA Loreto) placed a warning sign (right) at the swimming hole translated as 'swimming prohibited. Do not drink this water'

Table 1

Possible determinants of leptospirosis severity

Virulence properties of infecting Leptospira
Infectious inoculum at time of exposure
Preexisting immunity due to previous exposure
Hormonal influences (men more commonly develop severe disease)
Human host genetics: acquired or innate immunity
Nutritional factors: malnutrition, alcohol consumption