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LEPTOSPIROSIS IN MESOAMERICA (M GARCIA, SECTION EDITOR)

Challenges to Diagnosing Leptospirosis in Endemic Regions Require Urgent Attention

Rebecca S. B. Fischer[1] · **Byron Flores Somarriba**[2]

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Abstract
Purpose of Review Leptospirosis is one of the most widespread zoonotic diseases, poses health and economic threats across the globe, yet little investment in tools to identify and eliminate disease have been made.
Recent Findings Current gold standard diagnostics are time-intensive, suffer sensitivity and specificity challenges, and are scarce in resource-limited settings, where the largest disease burden exists. Central American countries are at higher risk than most of the world, although challenges to surveillance limit our understanding of the true impact of leptospirosis on that region. One of the greatest challenges to surveillance is the laboratory capacity and technical expertise to accurately and quickly diagnose disease.
Summary There is an immediate need for a redesign of the testing algorithm for leptospirosis in order to improve surveillance and inform treatment and prevention activities. A global collaboration to increase laboratory capacity in Central America that includes improved access to technologies beyond the current gold standard is important to explore.

Keywords Leptospirosis · Leptospira · Central America · Diagnostic techniques · Laboratory capacity

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✉ Rebecca S. B. Fischer
rebecca.fischer@bcm.edu

Byron Flores Somarriba
byronfloressomarriba@gmail.com

[1] Department of Pediatrics, Section of Tropical Medicine, Baylor College of Medicine, 1102 Bates Avenue Suite 550, Houston, TX 77030, USA

[2] Centro Veterinario de Diagnóstico e Investigación (CEVEDI), Escuela de Ciencias Agrarias y Veterinarias, Universidad Nacional Autónoma de Nicaragua-León (UNAN-León), Apartado Postal 68, Edificio Central, Contiguo a la Iglesia La Merced, León, Nicaragua

Introduction

Leptospirosis, a treatable and preventable disease, is one of the world's most widespread zoonotic diseases and poses health and economic threats across the globe, yet little investment in tools to identify and eliminate disease have been made [1, 2]. It is well known that the greatest burden of leptospirosis is borne by the world's impoverished populations, with the highest incidence occurring in tropical and subtropical regions [2–4]. A systematic review by Pappas et al. highlights Mesoamerica as one of the world's highest risk regions but also posited that data for some countries suffer from surveillance limitations, suggesting the situation may be more dire in that part of the world than has been documented so far [3]. In resource poor countries, surveillance is particularly difficult, in part due to challenges with healthcare utilization, disease diagnosis, and surveillance system adequacy; thus, the actual extent of leptospirosis in the highest risk areas is poorly understood [1, 5–8]. One of the greatest barriers to surveillance and disease diagnosis is the limited resources available for diagnostic capacity, and low-resource, tropical settings bear both the greatest risk of disease and endure the greatest challenges to surveillance capacity [2, 9]. In this age of prioritizing emerging and re-emerging tropical disease surveillance, there is a serious need for robust diagnostics for diseases like leptospirosis.

Pathogenic *Leptospira* reside most often in the renal tubules of host animals, infecting humans incidentally [7, 10, 11], while saprophytic *Leptospira*, which are considered non-pathogenic, live primarily in the environment. Since leptospirosis is primarily

transmitted via urinary excretion of leptospires by an infected or colonized animal host into the environment and subsequent acquisition by another human or animal host (by ingestion, through mucous membranes or abrasions, or by skin penetration), pathogenic *Leptospria* are present in the environment for some time [2, 11, 12]. And with animals serving as maintenance reservoirs for human disease-causing leptospires, their ambient environments are also transmission reservoirs.

It is important to distinguish pathogenic and non-pathogenic *Leptospira* for three vital reasons: (1) environmental specimens can contain pathogenic species, (2) clinical specimens can contain saprophytic species (through contamination or incidental host acquisition), and (3) not all *Leptospira* strains that cause disease in animals are pathogenic to humans, and vice versa (more *Leptospira* strains are pathogenic to humans than animals, and animals are more likely to harbor commensal strains) [7, 11, 12]. These are necessary considerations in characterizing infection status, and diagnostic models should include a means to distinguish non-saprophytic *Leptospira* and those that cause disease, taking into account the host species. A second need of a testing algorithm is to complement pathogen detection with antibody-based evidence of infection [9]. Lastly, the ability to discern specific phenotypic and genotypic types of *Leptospira*, the possibility of isolating and characterizing new strains, and the means to document changing characteristics of existing strains (e.g., acquisition of virulence factors) would greatly enhance our epidemiologic understanding of leptospirosis and facilitate outbreak detection and response.

Clinical Diagnosis

Clinical diagnosis of leptospirosis is difficult and has limited reliability, since symptoms are often non-specific, variable, and are easily classified as other febrile illnesses [7, 10, 11, 13, 14]. The low sensitivity and specificity of clinical diagnosis mean that sometimes only severe cases or those exhibiting a classic presentation will be diagnosed. Where regional case definitions are standardized, they may be insensitive to the changing local epidemiology of *Leptospira*. For example, diagnosis during outbreaks of emerging or rare strains may miss cases when symptoms and other clinical indicators are unusual for the region.

Asymptomatic individuals present another important challenge to recognition of leptospirosis, but they are an important population because both humans and animals could still be maintaining and shedding *Leptospira* even in the absence of overt disease [7, 12, 15–17]. As many as 70% of outbreak-associated human cases in Central America could be asymptomatic [16], and leptospirosis there may account for 6% of febrile illness attributed to other causes [14].

Laboratory Diagnosis

Specific microbiology is necessary for a confirmed leptospirosis diagnosis. Direct detection of the organism or its components (e.g., culture, microscopy, molecular techniques) and serology (e.g., microagglutination test (MAT), enzyme-linked immunosorbent assay (ELISA), and rapid diagnostic tests (RDTs)) are available. The gold standard diagnosis is culture from clinical specimen (urine, blood, or CSF) or MAT, the reference test. However, these are tedious, time-consuming and require carefully trained personnel and properly equipped laboratories. In reality, very few laboratories in Central America conduct these reference tests.

Characteristics of *Leptospira* and the host's immune response drive the utility of each [7, 18–20]. The timing of clinical specimens is important. The incubation period is variable, as long as 29 days, from the time of exposure to symptom onset [19, 21]. Leptospires may be present in the blood within the first 2–10 days after symptoms begin, declining until day 15, and in the urine after day 10. Conversely, antibodies appear later, typically with IgM present within 3–30 days and persisting for up to a year. IgG may develop during days 10–14 but, in some people, never appear, and a failure for *Leptospira* to elicit any antibody response at all has been reported. False negative results can be a product of testing a specimen outside of the ideal timeframe (such as antibody testing too early after infection or culturing a late phase blood specimen), and leptospires may not be detected if the patient has taken antibiotics, there is only a weak leptospiremia, or samples are poorly handled. An abnormal antibody response in some patients may preclude serology or limit the ability to attribute the response to a current infection (e.g., if IgM persists across multiple epidemics).

Direct Observation of *Leptospira*

Direct microscopy of leptospires in clinical specimens would be the simplest and most accessible test, but darkfield microscopy is required, and its interpretation is subjective. Proteins and other fibers in samples are easily confused for spirochetes, and leptospires cannot be distinguished from other spirochetes by microscopy. *Leptospira* of different species, pathogenic and non-pathogenic alike, are morphologically similar, with often only very slight differences between strains; morphology is not a useful tool for discerning whether a recovered isolate is disease-causing or not. Despite special staining techniques, the sensitivity and specificity of microscopy remain low.

Although a positive culture of *Leptospira* yields a definitive diagnosis of current infection or colonization, specimen collection and the culture process (e.g., during preparation or maintenance of special culture media or inoculation) allow numerous opportunities for contamination. This is particularly a problem in

resource-constrained laboratories, where sterilization techniques might not always be ideal and laminar flow hoods may be unavailable or not maintained. The technique requires specialized media and is time-consuming. Cultures, which can take up to 3 months to grow, are examined by darkfield microscopy to confirm spirochete morphology [19]. Since culture is not specific for pathogenic *Leptospira*, further tests would be required to classify the spirochete as *Leptospira* and distinguish if the culture represents an infecting isolate (rather than, using testing of animals as an example, a commensal non-pathogenic organism).

Microagglutination Test

MAT, developed a century ago, remains the reference antibody test [18, 20]. Since MAT is capable of distinguishing between *Leptospira* serogroups, it has historically been useful for classifying isolates as pathogenic or not. MAT reacts patient antibodies with *Leptospira* serovars known to be circulating in the region and serovars more broadly representative of all the serogroups known in the region (particularly valuable for endemic regions prone to changing epidemiology), and then examining via darkfield microscopy. A panel of reference strains must be maintained in live cultures, free from contaminants, and challenges to maintaining clean cultures were mentioned above. For MAT to achieve optimal test outcomes, some knowledge of the molecular epidemiology in the region is therefore required. In recent years, the ability of MAT to correctly assign serovars has been challenged [18, 20, 22, 23].

Interpreting MAT depends on assessing patient antibody titers at various dilutions and, ideally, comparing them to reference titers and serial clinical specimens from patients [20]. To achieve adequate sensitivity, antibody titer cut-offs are determined locally. High cut-offs are set in highly endemic countries, typically a 4-fold rise in antibody titer between acute and convalescent specimens, or a 1:≥800 titer for an acute specimen in the absence of a convalescent sample, and higher cut-offs have been recommended in hyperendemic countries [19, 24]. Additional complications of MAT interpretation are the test cannot distinguish between IgM and IgG; interpretation of the level of agglutination is subjective; an anamnestic antibody response to a previously infecting serovars may produce a high titer; a high degree of cross-reactivity between different serogroups makes actually assigning serovars difficult; the inability to discern between antibodies produced as a result of receiving a vaccine and those produced by a natural infection itself; it is likely to misidentify or assign false positives in the event of new serovars; confounding effect of multiple serovars coinfections; inconclusive results if a convalescent sample is unavailable; and failure to confirm infection if the clinical specimen was drawn outside of the antibody response timeframe [10, 19, 20].

Molecular Detection

Real-time polymerase chain reaction (PCR), known for its high specificity and sensitivity, can produce quantitative results (qPCR) and involves less time and less chance of erroneous interpretation, compared to direct culture and serologic characterization. It can be used with any specimen type and is an ideal rapid and early test of infection. In fact, detection of leptospires in an early clinical specimen would negate the need for time-intensive culture and serology. However, it requires specialized equipment (thermocycler), reagents, and technical expertise. In resource-constrained settings—importantly, exactly where the technique would afford the greatest advantage—these requirements pose a barrier to its use. Previous studies have demonstrated the usefulness of PCR to detect pathogenic leptospires in patients with negative culture results, supporting its usefulness as a complementary test and promoting its advantages to understanding leptospirosis [9, 24, 25, 26•, 27]. The article by Flores Somarriba et al., printed in this same issue, strengthens the a case for the implementation of molecular techniques to detect *Leptospira*, describes additional considerations and technical details of various qPCR procedures proposed for detection of *Leptospira*, and also discusses the advantages and challenges of Loop Mediated Isothermal Amplification (LAMP), a newer sequencing method that can be done in the absence of a cost-prohibitive thermocycler, and MLST, which uses seven genes to assign specific genetic classifications to isolates.

Other Diagnostics

In order to overcome the limited availability and complex nature of traditional laboratory tests, other simple diagnostic tests have recently become available for human and veterinary use. Some examples are commercial ELISA kits to detect IgM and IgG, indirect fluorescent antibody (IFA) IFA), immunochromatographic tests to identify antigens in human and animal urine using monoclonal antibodies, IgM dipstick tests, recombinant LipL32 proteins dipsticks for IgG, indirect hemagglutination (IHA), and latex agglutination (LA) tests [19, 28–34]. Agglutination-based testing is useful in regions with low economic and human resources, but it is recommended that each country or region validate the technique using. In practice, diagnosis of leptospirosis is primarily based on serology, and some countries have even developed their own serologic tests. However, serological techniques other than MAT are proposed only for screening purposes, with use of reference tests for confirmation. Interpreting any serological result should consider the timing and variability of host antibody responses mentioned above, and serology cannot definitively identify the infecting serovars.

Conclusion

Understanding the epidemiology of leptospirosis in resource-limited areas is hindered, and surveillance suffers from a scarcity of tests to confirm diagnosis. Even in resource rich settings, leptospirosis proves challenging to accurately define; where resources are constrained, a lack of diagnostic and technical capacity is a barrier to understanding the burden of disease, characterizing the local clinical presentations for endemic and epidemic serovars, delivering adequate treatment, and ultimately curbing morbidity and mortality. Understanding disease in animals is equally as important as understanding human disease, since animals are the primary maintainers and transmitters of leptospirosis and because the economic importance of animals in low income countries makes animal health important [8, 35•]. This lack of diagnostic laboratory services in the most heavily affected communities means that cases may never be confirmed or may be incorrectly classified, so the incidence of leptospirosis is severely underestimated in those communities. Of the 12 countries with the highest-ranked incidence of leptospirosis in the world, 3 are in Central America—Costa Rica (5th), El Salvador (8th), and Nicaragua (12th), establishing that continent as one of the highest risk regions overall [3]. Overlaying that scenario by the fact that four of the seven most impoverished nations in the world (Guatemala, Nicaragua, Honduras, and El Salvador) are in the same region of the world [35•] makes evident the crucial need in that region.

In this modern era of advancing technology, a more thorough diagnostic algorithm could more accurately characterize the epidemiology of leptospirosis, facilitate the detection of and rapid response to outbreaks and, at the same time, establish the molecular epidemiology of *Leptospira* where it is needed the most. Clinical diagnosis is the only option in most cases, but it is unreliable. Gold standard tests, which are based on an antiquated framework and are technically challenging to conduct and interpret, are only possible in a handful of laboratories. While bolstering access to MAT would be a step in the right direction, there is a real need to revisit the diagnostic algorithm to establish a multifaceted approach that could (1) combine pathogen detection methods and antibody-based evidence to assign infection status, (2) distinguish pathogenic from non-pathogenic leptospires in specimens, and (3) discern specific genetic characteristics of infecting *Leptospira*, as well as allow cases to have variable clinical presentations (including asymptomatic or very mild symptom manifestation). It is also prudent that the approach be able to detect known circulating types but also be conducive to identifying novel types and monitoring changing virulence properties.

Finally, reaching the goal of truly making testing widely available for clinical and research use requires looking forward to address the clear and pressing need for increased laboratory capacity (e.g., laboratory space, equipment, and technical expertise) and maintenance (e.g., access to supplies and reagents, servicing of equipment, proper storage and transport of specimens, best laboratory practices, and quality control) in settings with limited resources. The United Nations prioritized capacity building and investment in scientific research, specifically in developing countries, outlined in the 17 Sustainability Goals of their 2030 Agenda for Sustainable Development [36]. Even basic laboratory needs are scarce in Central America, and reference laboratories would not only benefit from more advanced technology, but are crippled without it. As emphasized by the Flores Somarriba group in Nicaragua, there are new strategies that can be feasibly implemented with the right support. Increasing capacity in this context will require a global effort by humanitarian organization, scientific associations, content experts, and others in collaboration with local leadership to mobilize financial resources and explore partnerships for transfer of technology [3, 37•]. Increasing the technical and intellectual capacity for diagnosis and surveillance in areas where *Leptospira* and other agents threaten the health of the world's disadvantaged is an urgent public health need.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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