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REVIEW

LEPTOSPIROSIS, A CLINICAL UPDATE REGARDING A NEGLECTED INFECTIOUS DISEASE

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ABSTRACT

Leptospirosis is one of the neglected infectious diseases locally widespread and extremely significant in tropical areas due to its great epidemic potential. It is a worldwide public health problem in view of the emergence and reemergence of the disease due to lack of sanitation and negligence, among other factors. In fact, leptospirosis infects more than 1 million people per year, resulting in almost 60,000 deaths. Human infection generally takes place after skin exposure to soil and/or water contaminated with urine of chronically infected mammals. The clinical presentations of the disease vary from a slight fever, goosebumps and flu-like symptoms to the acute forms of the disease. Understanding the main aspects of the disease is paramount due to the many unspecific signs and symptoms as well as frequently mistaken diagnosis. In this article, we discuss the epidemiological, immunopathogenic, clinical and prophylactic aspects of this condition with the purpose of clarifying an up to date panorama of the subject.

KEY WORDS: Leptospirosis; *Leptospira*; concepts; clinical update.

INTRODUCTION

Leptospirosis is a worldwide zoonosis particularly endemic in tropical countries. In fact, the incidence in tropical countries is 10 times greater when compared to temperate regions (Wang et al., 2020). However, climatic changes and global warming may alter this scenario, resulting in more favorable conditions for the transmission of the disease in other parts of the world. Furthermore, leptospirosis is reemerging in some European countries (Taniguchi & Póvoa, 2019).

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The disease is transmitted to humans by rodents, who are chronic carriers of leptospirosis and permanently excrete a significant number of bacteria into the environment in their urine, which maintains the microorganism in the environment (Haake & Levett, 2015a). The clinical presentation in humans may vary greatly, ranging from subclinical cases to cases that evolve with serious complications and may result in death. Normally, the initial characteristics of the condition include sudden fever accompanied by tremors, headache, conjunctivitis and intense muscular pain, symptoms that should draw doctors' attention to the suspected diagnosis. Some severe forms of the disease may present jaundice and renal failure (Karpagam & Ganesh, 2020).

Although leptospirosis affects all age groups and genders, and may be transmitted regardless of the season, it is frequently seen during the summer (in tropical areas), due to pluviometric volume and in the fall (in regions with a temperate climate), as well as in young male adults (Goris & Wagenaar, 2020). Individuals whose occupations require working in mining, fishing, poultry, agriculture and slaughterhouses or require veterinary activities are the most exposed and are more likely to contract the disease (Karpagam & Ganesh, 2020). In cities, porters are often affected since they are usually given the task of cleaning water tanks, instead of hiring specialized firms. Despite the reports and the historical importance of leptospirosis, the disease is considered re-emergent and neglected, and of global public health importance due to its high human and animal morbidity and mortality rates. It still emerges as a major cause of illnesses with acute febrile symptoms in many developing countries, a fact usually related to unplanned urbanization, poor sanitation and neglect (Karpagam & Ganesh, 2020). Therefore, this review covers the main aspects related to leptospirosis.

THE *Leptospira* GENUS

Leptospira are thin, spiraled, mobile and millimetric bacteria that feature "hooks" on both extremities (Karpagam & Ganesh, 2020). They are also Gram-negative and included in the Spirochaetales order and Leptospiraceae family (Goris & Wagenaar, 2020). These bacteria are strictly aerobic and utilize fatty acids and alcohol as a source of energy. The culture medium is specific, produced from rabbit or cattle serum (Fletcher or Stuart), requires darkness and grows at approximately 30ºC. The growth may be gradual and varies from five days to two months (Murray et al., 2017). Unlike other important spirochetes, such as *Treponema* and *Borrelia*, *Leptospira* feature lipopolysaccharides (LPS) on their surface, a characteristic related to pathogenicity. Other virulence factors include external membrane proteins, hemolysins, surface proteins and adhesins (Karpagam & Ganesh, 2020).

Leptospirosis is transmitted to humans through direct or indirect contact with urine from chronically infected carrier animals (Figure).

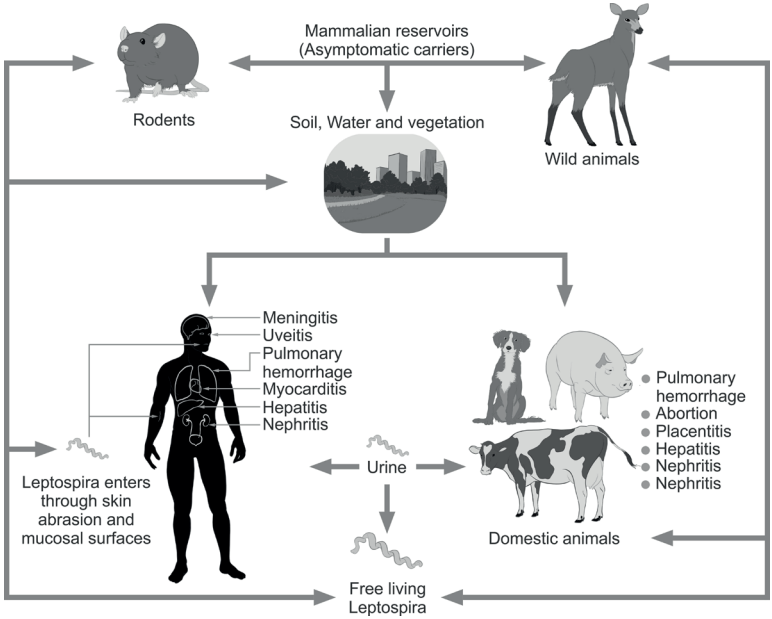

Figure. Forms of leptospirosis infection (Adapted from de Faisal et al., 2012)
Rodents and other wild animals are the main reservoirs of the pathogen and generally do not develop the clinical disease. *Leptospira* can reach soil, water and vegetation and from there infect humans and other mammals, penetrating through damaged skin, mucous membranes and conjunctival mucosa causing the disease.

Rodents, who are the main known reservoirs, rarely develop the clinical disease due to the interaction between the etiological agent and their renal system, which, based on its alkaline pH and renal histology, allows the dissemination of the bacteria to the environment by continued bacterial excretion through urine. The immune mechanism that explains this interaction is not yet fully understood, but it is known that renal alterations, such as proteinuria and decreased Tamm-Horsfall protein, are found in the urine of infected rodents (Ramachandrarao et al., 2015; De Brito et al., 2018).

Leptospirosis clinical cases can start out like flu, leading to multiple organ failure, possibly causing death (Karpagam & Ganesh, 2020). The severity of this disease depends on pathogenicity and the bacteria load along with the patient's immunologic status (Goris & Wagenaar, 2020).

The pathophysiology of the condition can be explained by a few mechanisms and is dependent on the bacteria's direct action, the patient's humoral immune response (capable of determining the type of lesions that will be triggered) (Haake & Levett, 2015a) and the presence of an endotoxin that promotes endothelial lesions and cell-degenerative processes (Chin et al., 2018). In turn, the endotoxin, is possibly involved in the development of lesions and clinical manifestations of different degrees of severity (Diament et al., 2002; de Albuquerque et al., 2012).

Leptospira, being mobile, are capable of invading skin damaged by abrasions, the mucous membranes of the conjunctiva or the oral cavity, generating infection (Haake & Levett, 2015b). With the invasion of tissue and the vascular system, damage occurs in the endothelium of small vessels, causing most of the symptoms in the septicemic phase which manifests in the first few days of the disease (Haake & Levett, 2015a). The presence of the bacteria in the blood causes an inflammatory reaction of the capillaries, leading to an increase in capillary permeability, due to endothelial barrier disfunction, and in severe cases, hemorrhagic diathesis and the loss of tecidual oxygenation (Rezende et al., 1997). In other words, the damage to the cell membrane leads to a loss of vascular integrity causing ischemia and necrosis of the cells (De Brito et al., 2018). Some experiments found that *Leptospira* antigens are able to bind to the capillary endothelium, to cells located in the tubular epithelium and to macrophages, indicating their important role in leptospirosis' immuno-pathogenesis (Pereira et al., 1997). *Leptospira* colonize and persist in the kidney's proximal tubule, which can result in chronic tubulo-interstitial inflammation, as well as fibrosis (Hung, 2019). Human necropsies evidenced the presence of *Leptospira* antigens in the pulmonary tissues of patients that died after contracting the pulmonary form of the severe disease, indicating that the cellular elements were related to the pathogeny of this manifestation (Pereira et al., 2005).

The immune response against *Leptospira* depends on the production of specific antibodies against LPS as well as the activation of the complement system's classic pathway. It has been demonstrated in experimental models that the effective phagocytosis of these bacteria, performed by neutrophils and macrophages, depends on the IgG-specific opsonization (Fraga et al., 2011). The LPS located on the *Lesptospira's* membrane activate human macrophages against the toll-like receptor 2 (TLR2) (Hung, 2019).

Hemolytic toxins, produced by the bacteria, can act like sphingomyelinases and phospholipases and cause damage to surrounding tissue (Haake & Levett, 2015a), stimulating a humoral immune response and the production of IgM antibodies that neutralize the antigens and prevent the microorganism from being detected in biological fluids, with the exception of urine. The immune phase then begins, in which it is possible to observe the variation in the number of T lymphocytes, with a reduction of $CD3^+$ and of $CD4^+$, and the multiplication of B lymphocytes (Yamashiro-Kanashiro et al., 1991). This phase can last up to a month and is characterized by the involvement of different systems and multiple organs, proportional to the host's immunological reaction and the damage suffered in the septicemic phase. Some experiments indicated that the alterations in T $CD4^+$ and T $CD8^+$ lymphocytes are linked to a more severe evolution of the disease, with renal and pulmonary damage, confirming the hypothesis that these cells are fundamental in the host's immunologic reaction (da Silva et al., 1998).

Recent studies demonstrated that the superficial proteins of the pathogenic *Leptospira* species, known as Lp25 and Lop32, are associated with acute clinical presentations of hypokalemic pre-renal failure found in patients with rhabdomyolysis. The first protein causes diffuse and more severe damage to the renal parenchyma and the second protein causes damage with milder and focal characteristics, thus explaining the interstitial-tubular nephritis found in this disease (De Brito et al., 2018). Bleeding is caused mainly by diffuse capillary damage. Other factors, such as decreased platelet count or hepatic dysfunctions, despite aggravating the clinical situation, have less expressive roles in theses hemorrhagic disorders (CDC, 1997).

Jaundice is the primary consequence of the increase in conjugated bilirubin, enzymatic damage at hepatocyte level and of intra-hepatic cholestasis, which all lead to the inadequate excretion of this metabolite (Wang et al., 2020; De Brito et al., 2018). It is believed that this cholestasis is caused by the *Leptospira* toxin, capable of provoking injuries to the cell membrane so that the bacteria relocates itself from the sinusoidal capillaries to the biliary capillaries, due to a cell junction rupture. Thus, the bile overflows from the capillaries to the blood, inducing jaundice (De Brito et al., 2018).

Cardiac repercussions are related to electrolyte disorders, especially the decrease of potassium, elevation of cardiac troponin I and increased uremia (Jayathilaka et al., 2019). These alterations are repercussions of conditions such as arrhythmias, pericarditis (Farrar, 1995) and myocarditis (Goris & Wagenaar, 2020), and, eventually, from injury to the muscular layers of the heart (Herdy et al., 1993). Hematological complications are rare and include hemolysis, thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome (Goris & Wagenaar, 2020).

NATURAL PROGRESSION OF THE DISEASE

Leptospirosis symptoms are extremely diverse, ranging from asymptomatic cases and self-limited febrile illness to fatal cases of kidney injury and bleeding. For a more didactic approach, the natural progression of the disease will be divided into two extreme scenarios: the non-icteric form and the icteric form (Wang et al., 2020).

NON-ICTERIC FORM

The majority of cases have mild manifestations with sudden fever, headache, asthenia, loss of appetite, nausea, vomiting, muscular pain and diarrhea. These symptoms can be confused with a flu-like syndrome (Wang et al., 2020), amounting to approximately 90% of the cases. Generally, the fever exceeds 40ºC (Vinetz & Watt, 2020). Not all of those infected will manifest cutaneous lesions, but cases presenting cutaneous alterations will have lesions with maculo-papular characteristics, erythema or will present petechiae (Rezende et al., 1997). Conjunctival suffusion is a useful diagnostic finding, observed in 30% of patients, and usually appears within two or three days after the onset of a febrile episode and involves conjunctival edema (Vinetz & Watt, 2020).

The symptoms can last up to a week and, subsequently, the patient may heal or proceed to the immune phase after the defervescence period. In this phase, the main findings are ocular and neurological disorders. *L. interrogues* serotype autumnalis can cause distinct erythematous pre-axial lesions (Mégraud et al., 2017). Among the alterations found in the central nervous system, meningitis, presenting transparent fluid, is the most common (Romero et al., 1998; Schillinger et al., 1999). Patients with this manifestation usually develop signs of meningeal inflammation, confusion, hallucinations and even delusions. There are some reports of other neurological manifestations, such as facial paralysis (Costa et al., 2001), Guillain-Barré (Bal et al., 2003) and myelitis (Rezende et al., 1997).

Another possible finding is uveitis, which appears as a late manifestation of leptospirosis, and may be uni or bilateral, with hypopyon and retinal periflebitis, which generally leads to certain recovery. The ophthalmological manifestations are related to the presence of bacteria in the anterior chamber of the ocular globe (Chu et al., 1998).

ICTERIC FORM

The icteric form of the disease is more likely to lead to complications, such as acute kidney injury, cardiovascular alterations, hemorrhages and the involvement of the pulmonary system, resulting in high mortality rates (Vinetz & Watt, 2020).

In the icteric form, the patient complains of intense muscular pain, especially in the calf, tending to appear between three to seven days after the onset of the symptoms. The jaundice has a peculiar characteristic, being of an orange hue, which is why the icteric syndrome observed in leptospirosis is known as rubinic jaundice (Brasil, 2014).

When visceromegalies are found, these occur at the expense of the liver and spleen (Day, 2019). In the case of hepatic disorders, alterations in the level of transaminases and a slight elevation of the alkaline phosphatase levels can be observed in the course of the disease (De Brito et al., 2018). There is an intense cutaneous vasodilation, and there may also be dehydration, shock, acute kidney failure and electrolyte, metabolic and acid-basic disorders (Day, 2019).

Eventual bleeding may occur as small petechiae or severe blood-loss through the gastrointestinal and pulmonary systems (Vinetz & Watt, 2020). The patient can develop anemia, present alterations in his/her electrocardiogram due to the impairment of the heart's electrical conduction system (De Brito et al., 2018), aortitis in 57% of all cases (which differs from the syphilitic since it does not obliterate vasa vasorum) and cardiac failure (De Brito et al., 2018; De Brito et al., 1987).

An important triad of symptoms is common to this disease — known as Weils syndrome, it is characterized by the presence of jaundice, acute kidney injury and hemorrhage, and indicates a poor prognosis (Rathinam & Namperumalsamy, 1999). Some infectious conditions, acquired in a hospital setting, such as sepsis, pneumonia and urinary tract infections may favour the patient's worsening (Vinetz & Watt, 2020).

LABORATORY DIAGNOSIS AND TREATMENT

The treatment should not wait for laboratory confirmation. The diagnosis should be based on clinical suspicion and potential epidemiological exposure (Haake & Levett, 2015a). However, the detection of antibodies may be performed by means of serological tests or by isolating the bacteria in a culture medium (the latter usually performed in specialized centers) (Brazil, 2014). The diagnosis can also be reached by the demonstration of bacteria DNA with Polymerase Chain Reaction (PCR). Nevertheless, the laboratory method chosen depends on the evolutionary stage of the patient (Brazil, 2019).

The detection of *Leptospira* in fluids (blood, urine and cerebrospinal fluid) or tissues can be performed by direct microscopy with dark-field contrast. The culture requires an adequate medium such as EMJH (Ellinghausen McCullough Johnson Harris Medium) enriched with bovine serum albumin and Tween 80 or rabbit serum (Verma et al., 2020). The culture should be kept at a temperature of 28ºC to 30ºC, and growth can take place between 10 to 60 days (Murray et al., 2017). During bacteremia (the first ten days since the onset of symptoms), the etiological agent can be isolated in blood and in the cerebrospinal fluid (CSF), the latter indicated when meningitis is suspected (Brazil, 1999). The serological methods, used in the diagnosis of leptospirosis, enable detection of the disease after the sixth day of its onset using serum antibodies. In this context, the most common techniques are the enzyme-linked immunosorbent assay (ELISA) and the microscopic agglutination test, the latter being considered the gold standard (Verma et al., 2020).

Real time PCR has been used for direct detection of *Leptospira* (Esteves et al., 2018), with great effectiveness, especially when it is performed in the first days of infection due to its high sensibility and specificity (Tavares & Marinho, 2015).

Treatment using early administration of antibiotics may be effective in decreasing the severity as well as the duration of leptospirosis. Therefore, it is advisable to start antibiotic administration as soon as possible, even without the clinical laboratory results. Doxycycline is the first choice for patients with mild symptoms and the recommended dose is 100mg twice a day, administered orally. If it is contraindicated, an alternative is ampicillin or amoxicillin (Galloway et al., 2019).

In severe cases, intravenous penicillin can be administered (1.5 million IU every six hours) (Goris & Wagenaar, 2020), an alternative being ceftriaxone (Galloway et al., 2019). More severe cases may require hospitalization and supportive therapy, including intravenous hydration and electrolyte supplementation. In cases that evolve to kidney and respiratory failure, dialysis and mechanical ventilation should be included, respectively (Goris & Wagenaar, 2020; Galloway et al., 2019).

EPIDEMIOLOGY AND CONTROL

The notification of leptospirosis cases to health authorities is mandatory in Brazil (Brazil, 2016) and notification of new cases is essential for surveillance and control of any possible outbreaks of the disease (Brazil, 2019). Leptospirosis is an under reported disease, not only in Brazil, but worldwide, which can be attributed to the lack of clinical suspicion and low diagnostic ability. The diagnosis of the disease is truly difficult and initially mild cases are only recognized through the observation of the clinical presentation (Rodrigues, 2017).

EPIDEMIOLOGICAL CHARACTERISTICS

The pathogenic *Leptospira* are found widespread in nature, due to their continued presence in the kidneys of domestic and wild animal hosts (Haake & Levett, 2015b). The transmission of the disease through food contaminated with the host's urine or through aerosols is considered to be rare and the possibility of *Leptospira* penetrating intact skin could happen when skin portions remain under water for a period of time (Day et al., 2018).

Leptospirosis is spread worldwide, being endemic in Brazil, especially in capitals and metropolitan areas, a fact generally associated with floods and the agglomeration of low-income individuals. Other important factors are inadequate sewage systems and hygiene as well as the number of infected rodents (Brazil, 2019). Furthermore, the incidence of the disease is seasonal in nature, occurring in greater numbers in the summer due to flooding.

Rodent reproduction is controlled by the supply of available food, a direct consequence of the country's inefficient garbage collection method and other precarious socio-environmental factors, favoring the emergence of the disease as well as other common diseases normally found in underdeveloped countries (CDC, 1997). The prevalence is under reported in some regions of the world where complementary tests to confirm the disease are not often available, such as the Pacific islands (Guenier et al., 2018). The person-to-person form of transmission is not usual (Brazil, 2014). Leptospirosis is less frequently observed in developed countries where professional and occupational contact are the most important epidemiological factors (CDC, 1997).

The infection in rodents, such as *Rattus norvegicus* species, is normally asymptomatic and occurs through the colonization of their renal tubules as well as the consequent elimination of bacteria through their urine contaminating the environment and humans (Haake & Levett, 2015a). However, canine, feline, swine and equine infections can be present with the systemic form of the disease (Adler & Dela Peña, 2010) and spontaneous abortions in female cattle, swine and goats are a direct and common result of the infection (Day et al., 2018).

The persistence of *Lepstospira* in the soil is influenced by the soil's intrinsic physicochemical characteristics, such as humidity, pH, organic content and texture (Casanovas-Massana et al., 2018), and tropical and subtropical climates are the most suitable for bacterial survival (Goris & Wagenaar, 2020). In the transmission cycle of the disease, humans are incidental hosts that can become infected through contaminated water, occupational activities with exposure to sick animals and after contact with floodwater (Haake & Levett, 2015a).

The minimum incubation period is one day, and can be extended up to 30 days with an average of four to fourteen days (Brazil, 2019). Leptospirosis can affect both sexes and any age group. However, most cases are seen in males, and incidence peaks occur in the summer and autumn, both in the Northern and Southern hemispheres, and in the tropics during the rainy season. It is estimated that approximately one million severe cases occur annually worldwide, and the average lethality rate reaches almost 10% (Goris & Wagenaar, 2020). Weil Syndrome, the most severe form of leptospirosis, presents a lethality rate that ranges from 5 to 15%, and typically develops with kidney and liver failure (Galloway et al., 2019).

According to the Brazilian Ministry of Health, there are cases of leptospirosis in all states of the country, presenting higher prevalence in the southern and southeastern regions. From 2000 to 2017, 66,090 cases were notified and, according to the National Disease Notification System (SINAN), 6.295 deaths due to complications were reported. In other words, the lethality rate of the condition in the country is around 9% (Brazil, 2017).

PROFILAXIS AND CONTROL

The highest risk of exposure to *Leptospira* is related to occupations or recreational activities in fresh water (Brazil, 2019). Prophylactic methods include avoiding exposure to urine and infected animal tissue, and if animal handling is required, personal protective equipment should be utilized. It is also important to consider rodent population control, the natural reservoir of this disease (Goris & Wagenaar, 2020; Brasil, 2019).

Vaccines for livestock animals are available but special attention should be paid to the serovars present in each region (Goris & Wagenaar, 2020), already isolated in cases of leptospirosis in humans in Brazil. Vaccination can decrease the incidence of the disease in domestic animals and production livestock, without altering the incidence in humans. Nevertheless, a number of immunized animals are infected and perpetuate the elimination of the microorganism in their urine, contaminating the environment (Day et al., 2018). In European and Asian countries, human vaccination against a specific prevalent serovar has been performed and has shown to be effective (Goris & Wagenaar, 2020).

Currently, the available vaccines are composed of inactivated *Leptospira* in formalin, but generally have limited efficacy, due to their inability to stimulate cross-protection against different serovars, and have potentially serious side effects. Therefore, more recent studies have concentrated their efforts in the search for a peptide and subunit vaccine, since these contain specific immunogenic components of the pathogens which cause infection. In the search for more effective vaccines, immunoinformatic screening has been shown to be a promising strategy and research has been carried out to identify

conserved immunogenic proteins from the *Leptospira's* outer membrane, in addition to identifying the epitopes of B cell, cytotoxic T lymphocytes and helper T lymphocytes (Lata et al., 2018).

In spite of debates, chemoprophylaxis with doxycycline (200 mg, once a week) or azithromycin (in pregnant women and children) is indicated solely during pre-exposure and post-exposure, in short exposure situations (Goris & Wagenaar, 2020). Other strategies involve the proper management of urban waste, basic sanitation, the use of potable drinking water and population health education on data regarding the disease and prevention. Likewise, avoidance of still water that may contain *Leptospira* is also a recommended measure to prevent leptospirosis (Brazil, 2019).

CONFLICT OF INTEREST

There is no conflict of interest

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