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Leptospirosis: Transmission, Diagnosis and Prevention

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ABSTRACT: Leptospirosis is probably the most widespread and prevalent zoonotic disease in the world. It is difficult to diagnose both in the clinic and the laboratory. Therefore, the disease is frequently not recognized and consequently severely neglected. Leptospirosis is (re-)emerging globally and numerous outbreaks have occurred worldwide during the past decade. Leptospirosis affects humans in rural and urban settings and in industrialized and developing countries. The most recent examples are the epidemics in Nicaragua in 2007, in Sri Lanka in 2008 and in the Philippines in 2009 , each affecting several thousands of people and causing hundreds of deaths. Based on global data collection 300,000–500,000 cases of leptospirosis occur annually, and the annually death rate worldwide is estimated to be 58,900.

KEYWORDS: leptospirosis, Zoonosis, Morocco, Elisa, PCR, Prevention.

1 INTRODUCTION

Leptospirosis is a bacterial zoonosis that is common worldwide, especially in developing countries. Organisms are shed in the urine of infected animals, including rodents and domesticated animals, which may not show signs of disease. Humans usually become ill after contact with infected urine, or through contact with water, soil or food that has been contaminated [1]. Leptospirosis is caused by the spirochete Leptospira interrogans. There are over 200 pathogenic serovars, divided into 25 serogroups. Infection confers serovar-specific immunity, but further infections can occur with different serovars [2]. The sources of infection are infected animals [3]. The main transmission routes are through injured skin and via long periods of exposure to contaminated water or soil [4]. Leptospires can survive for weeks or months in the environment under favorable conditions, such as temperatures of 28°C to 32°C and a neutral or slightly alkaline pH [5].

Clinical manifestations of leptospirosis varies from mild to severe and symptoms include fever, myalgia, headache, malaise, intense jaundice and bleeding [6]. The most severe forms of it include Weil's disease, pulmonary hemorrhage syndrome and liver or renal failure may also develop and consequently lead to death [7]. Timely diagnosis of leptospirosis is essential for early and effective treatment. The major concern with leptospirosis diagnosis are its similarities to other diseases, which makes it difficult to differentiate from other febrile conditions like influenza, hemorrhagic fevers, typhoid fever, rickettsiosis, aseptic meningitides, hepatitis and malaria [8].

2 LEPTOSPIRA (THE ORGANISM)

Leptospires are spirochetes, and include both saprophytic and pathogenic species comprising the genus Leptospira, which belongs to the family Leptospiraceae, usually about 6-25 µm long and 0.1 to 0.2 µm in diameter, and have a typical double membrane structure as found in other spirochetes as it shown in Figure 1. Leptospires are highly motile, obligate aerobes, with an optimum growth at a temperature of 28 to 30 °C and at pH range 6.8 to 7.4. Leptospires can survive in a moist environment (e.g. soils, mud, swamps, streams and rivers), in organs and tissues of live or dead animals, or in diluted milk.[9,6].

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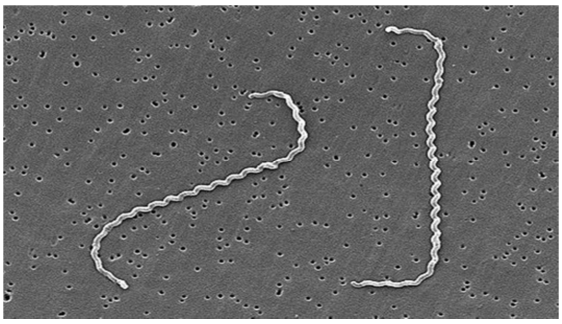

Fig. 1. Scanning electron micrograph of L. interrogans serovar icterohaemorrhagiae strain RGA bound to a 0.2-mm membrane filter. Reproduced from reference 625a with permission. Photo from Levett PN [12].

3 HISTORICAL ASPECTS

In 1882, Adolf Weil reported his description of a clinical syndrome characterized by splenomegaly, jaundice and nephritis, commonly referred to as Weil´s disease which became synonymous with leptospirosis. Leptospires were first identified as a cause of Weil´s disease in Japan, where it was common among coal miners [10].

4 EPIDEMIOLOGY AND BURDEN OF HUMAN AND ANIMALS

Leptospirosis occurs worldwide, except in polar regions. It is considered to be the most widespread zoonosis in the world [2,11]. The incidence is significantly higher in warm climate countries than in temperate regions, this is due mainly to longer survival of leptospires in the environment in warm, humid conditions [12]. Infected animals may have leptospires persistently colonising the proximal renal tubules and excrete the organism intermittently for months or years, or even for lifetime [5]. Disease is maintained by chronic carrier hosts that excrete the organism into the environment, and infection in man results from direct contact with infected animals or indirect contact with a contaminated environment[13].

Although excretion of leptospires in human urine for weeks or months, humans are not regarded as a source of transmission [14].

In animals the presence of Leptospira spp. in semen of infected bulls was demonstrated naturally and experimentally, indicating the possibility of bovine leptospirosis transmission by natural coition or by artificial insemination [15].

Global incidence of leptospirosis is still not well known [16]. The World Health Organization (WHO) has estimated that the annual incidence of leptospirosis is 0.1–1 case/100,000 people in temperate non-endemic areas, and 10–100 cases/100,000 people in humid, tropical, endemic areas [20]. The number of severe cases were reported to be approximately 300,000 to 500,000 each year worldwide, with case fatality rates of up to 30% [16,7]. Some authors indicated that the annually death rate due to leptospirosis worldwide is estimated to be 58,900 [17]. Environmental conditions strongly affect the transmission of leptospirosis. Leptospiral diversity is limited on islands such as Barbados, where only four pathogenic serovars infectious to people have been identified, and in urban environments where the major potential reservoir mammals are limited to rats and dogs [14].

In tropical regions with high species richness, such as the Amazon basin or other continental settings like rural southeast Asia, wild mammals would probably be infected by leptospires, and these leptospires should be highly diverse [14].

Besides the significant impact on public health, leptospirosis is also an animal health problem that causes economic losses in the livestock industries, due to reproductive failure, decreased milk and meat production, reduced growth, and clinical illness [11].

In addition young animals are generally more susceptible to the disease than adults and often suffering severe outbreak and high mortality rates [18].

An infected animal can remain symptom-free and shed infectious organisms in the urine for its entire lifetime [14]. A recent study of seemingly healthy dogs in Kansas, USA confirmed such an event. Of 500 dogs assessed without regard to health status, 41 were shown to have leptospiruria by PCR, and only four had clinical findings consistent with leptospirosis [14].

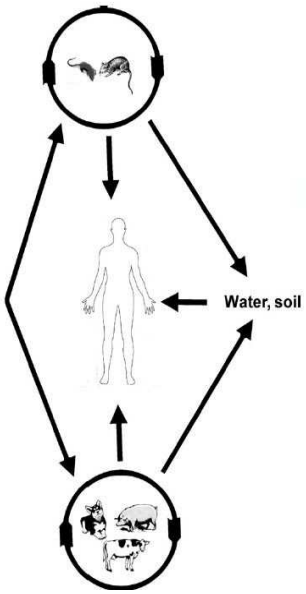

Fig. 2. Epidemiology of leptospirosis in animals and humans, photo from [6]

5 RESERVOIRS OF INFECTION

Leptospira has been isolated from virtually all mammalian species[11]. Numerous mammalian species are natural carriers (maintenance hosts) of pathogenic leptospires, including feral, farm and pet animals[12], but a significant source of infection for humans is the rat, which is associated with the more serious, icteric manifestation of leptospirosis[2], Infected animals may excrete leptospires intermittently or regularly for months or years, or for their lifetime [11].

Leptospira serovars are generally adapted to one or more mammalian. Dogs are reservoir hosts for serovar Canicola, and pigs for Bratislava and Pomona. Horses may also maintain Bratislava. Cattle are the primary reservoir hosts for Hardjo; however, this serovar is also maintained in farmed red deer (Cervus elaphus) and wapiti (Cervus elaphus nelsoni) in New Zealand, and sheep and goats may also have a role [1].

Rodents and insectivores are reservoir hosts for a number of Leptospira serovars. In particular, rats are important hosts for serovars Icterohaemorrhagiae and Copenhageni in the serogroup Icterohaemorrhagiae [1].

6 INCUBATION PHASE

In humans The incubation phase from exposure to onset of symptoms averages from 7 to 12 days, though it can be as short as 3 days or as long as a month [16].

In animals the incubation period can be as short as a few days, with clinical signs appearing after 5 to 15 days in experimentally infected dogs. It may be longer when clinical signs are the result of chronic, low level damage to the kidneys or liver. Rare case reports in cats suggest the possibility of prolonged incubation periods in this species [1].

7 CLINICAL MANIFESTATIONS

Leptospira infections may be asymptomatic, mild or severe, and acute or chronic. The illness tends to be milder in the reservoir host, and more severe when the serovar is not adapted to the host species [1].

In humans, severe leptospirosis is frequently but not invariably caused by serovars of the icterohaemorrhagiae serogroup. The specific serovars involved depend largely on the geographic location and the ecology of local maintenance hosts. Thus in Europe, serovars copenhageni and icterohaemorrhagiae, carried by rats, are usually responsible for infectious, while in Southeast Asia, serovar lai is common [12].

Acute or icteric disease could lead to death. In man it causes death to 25-50% of hospitalized cases, depending on the virulence of the infecting serovar. The acute disease shows, in addition to jaundice and haemoglobinuria, signs of meningitis, pulmonary hemorrhage and signs from complete systemic collapse due to kidney failure. If the acute disease is milder in its

clinical appearance, it shows flue-like signs and remains mainly undiagnosed. Man is seldom chronically infected. Chronic disease is mostly found among animals. Abortions and neonatal deaths are the common outcome of animal chronic infection. They are also of economic importance when food producing animals are infected [19].

In animals abortions usually occur 2 to 12 weeks after infection in cattle, and 1 to 4 weeks after infection in pigs [1].

7.1 CLASSIC SYMPTOMS

7.1.1 ANICTERIC FEBRILE ILLNESS

The typical leptospirosis is biphasic. The first septicemic phase lasts for 4-7 days and is characterized by acute systemic infection and leptospira in the blood and in cerebrospinal fluid. This is followed by a period of one to three afebrile and asymptomatic days and then the second immune phase starts with fever and leptospira in the urine and lasts for 4-30 days or longer. This bifasic course may not be seen in all patients.

Common symptoms are sudden onset of fever (typically 39°C) sometimes with rigors and chills, headache, muscle pain and tenderness, malaise with or without vomiting. Chest pain, dry cough and haemoptysis may occur. Some patients can develop mental symptoms of restlessness, confusion and delirium.

The most characteristic findings on examination are conjuctival suffusion and severe myalgia. Conjuctival suffusion is bilateral and usually associated with subconjuctival haemorrhage. Myalgia is most commonly located in the lower limbs and is so severe that even touching the muscle causes intense pain [20].

Aseptic meningitis may be found in 25% of all leptospirosis cases and may account for a significant minority of all causes of aseptic meningitis. Patients with aseptic meningitis have tended to be younger than those with icteric Leptospirosis. In one study authors noted that 62% of children ≤14 years old presented with aseptic meningitis, whereas only 31% of patients aged 15 to 29 years did so and only 10% of those over 30 years of age. Mortality is almost nil in anicteric leptospirosis but death resulting from massive pulmonary hemorrhage occurred in 2.4% of the anicteric patients in a Chinese Outbreak [12].

7.1.2 ICTERIC LEPTOSPIROSIS (WEIL´S DISEASE)

In some patients the septicemic phase progress to severe icteric illness with renal failure. Jaundice is the most important clinical feature of the severity of illness and is due to hepatorenal necrosis, intrahepatic cholestasis and increased bilirubin load from absorption of tissue haemorrhage. The liver is often enlarged and tender. Renal involvement is the most serious complication and is the most common cause of death in icteric leptospirosis. Oliguria can occur as early as the fourth day of illness but more often in the second week.

Meningeal symptoms are frequent but overshadowed by hepatic and renal features.

Severe bleeding, cardiac and pulmonary complications are frequent. Toward the end of the second week the patient is deeply jaundiced, ureaemic and haemorragic and become comatose. Death may occur in this stage due to renal failure and the mortality rate may be as high as 15-40%. In those who are not severely ill, recovery takes place in the second week [20].

7.1.3 OTHER COMPLICATIONS

Acute infection in pregnancy has been reported to cause abortion. In one of the cases reported by Chung et al. [21], leptospires were isolated from amniotic fluid, placenta, and cord blood; the infant was mildly ill and was discharged at 2 weeks of age. In another case, a neonate developed jaundice and died 2 days after birth [22], [12].

7.1.4 RECOVERY PHASE

With proper supportive care, most leptospirosis patients recover completely, patients with acute renal failure who require dialysis typically regain most of their renal function, although there may be evidence of persistent mild renal impairment, in addition, there is growing recognition that many patients suffer from chronic postleptospirosis symptoms. In a recent study of laboratory-confirmed leptospirosis patients in the Netherlands, 30 % of patients experienced persistent complaints after acute leptospirosis (PCAC) characterized by fatigue, myalgia, malaise, headache, and weakness, of patients with PCAC, 21 % reported that their complaints lasted for more than 24 months [21].

8 TRANSMISSION

Leptospirosis is a direct zoonosis. Rodents are considered as reservoirs host for leptospiral infection but domestic animals play an important role in the transmission [23]. Small mammals are the most important reservoirs [21]. Leptospirosis can be transmitted either directly between hosts or indirectly through the environment [1]. Human infection results from exposure to infected urine of carrier mammals [14]. Indirect contact with infected animals, via water or soil contaminated with infected urine, is a more common source for human infection than direct contact with infected animals [8].

The organisms usually enter the body through mucous membranes or abraded skin [1], and via long periods of exposure to contaminated water or soil [4].

Leptospires have been isolated from human breast milk. And in one case serovar hardjo was probably transmitted from an infected mother to her infant by breastfeeding [21].

In rare instances, human cases have also been transmitted during sexual intercourse. Other uncommon routes of exposure in people include rodent bites and laboratory accidents [1].

In animals the possibilities for transmission through direct contact within animal populations include sexual contact or artificial insemination [4].

Leptospira can be found in aborted or stillborn fetuses, as well as in normal fetuses or vaginal discharges after giving birth. They can be isolated from the male and female reproductive organs in some species, and these infections may persist for long periods. For example, serovar Hardjo may be found in the reproductive tract of both cows and bulls for more than a year [1].

9 RISK FACTORS

Individuals with occupations at risk for direct contact with potentially infected animals include veterinarians, abattoir workers, farm workers (particularly in dairy milking situations), hunters and trappers, animal shelter workers, scientists, and technologists handling animals in laboratories or during fieldwork. The magnitude of the risk depends on the local prevalence of leptospiral carriage and the degree and frequency of exposure [24]. Agricultural workers at risk for leptospirosis include rice field workers, taro farmers, banana farmers, and sugar cane and pineapple field harvesters [12].

These occupations involve activities likely to result in exposure of cuts and abrasions to soil and water contaminated with the urine of rodents and other animals attracted to food sources. For example, banana workers accounted for two-thirds of the reported leptospirosis cases in a tropical region of Queensland, Australia [25].

Most of these infections are preventable by the use of appropriate personal protective equipment such as rubber boots, gloves, and protective eyewear [24].

Recreational exposures include all freshwater water sports. The importance of this type of exposure has increased over the past 20 years as the popularity of adventure sports and races has increased, and also because the relative cost of travel to exotic destinations has decreased [26]. Urban slum dwellers in areas with poor sanitation are at particularly high risk [10].

These factors are most likely surrogates for rat exposure, as proximity to uncollected trash and sighting of rats increased the risk of leptospirosis among residents of urban slums [27].

Significant exposure also occurs from normal daily activities, with high rates of infection during heavy rainfall and flooding. In developed countries, infection is now increasingly being associated with outdoor recreational exposure and international travel [28].

9.1 RAINFALL AND FLOODING

Heavy rainfall and flooding increase the risk of leptospirosis by bringing bacteria and their animal hosts into closer contact with humans. Numerous outbreaks of leptospirosis have been reported following extreme weather events around the world, in geographically diverse areas. In Argentina, flooding has emerged as the major risk factor for leptospirosis, ahead of occupational exposure. In Mumbai, India, an eight-fold increase in disease incidence was noted after severe flooding in 2005. In Manila, Philippines, a large outbreak of leptospirosis was reported after tropical storms and severe flooding in October 2009.

A higher seroprevalence of infection has also been associated with heavy rainfall and flooding in China, France, Brazil, Trinidad and Tobago, and French Polynesia [28].

9.2 POOR SANITATION AND INADEQUATE WASTE DISPOSAL

Sanitation and waste management are major problems in developing countries and contribute significantly to the incidence of many infectious diseases and other adverse health outcomes. The presence of garbage, waste and sewage encourage the proliferation of rodents and can therefore increase the risk of leptospirosis. Garbage can also block drainage systems and exacerbate flooding risk. Many studies around the world have confirmed that close contact with garbage and sewage are significant risk factors in leptospirosis transmission, particularly in urban slums [28].

10 PREVENTION

Prevention of leptospirosis may be achieved by avoidance of high-risk exposures, adoption of protective measures, immunization, and use of chemoprophylaxis, in varying combinations depending on environmental [29]. Circumstances and the degree of human activity. High-risk exposures include immersion in fresh water, as in swimming, and contact with animals and their body fluids [29].

Removal of leptospires from the environment is impractical, but reducing direct contact with potentially infected animals and indirect contact with urine-contaminated soil and water remains the most effective preventive strategy available. Consistent application of rodent control measures is important in limiting the extent of contamination. Appropriate protective measures depend on the activity, but include wearing boots, goggles, overalls, and rubber gloves. In tropical environments, walking barefoot is a common risk factor [30].

Moreover, although these vaccines prevent against disease, they do not prevent infection and renal colonization; thus, they have little effect on the maintenance and transmission of the disease in the animal population in which they are applied [31].

Human immunization is not widely practiced. A vaccine containing serovar Icterohaemorrhagiae is available in France for workers in highrisk occupations, and a vaccine has been developed for human use in Cuba [32].

The use of doxycycline prophylaxis after excess rainfall in local populations in endemic areas has been studied [33]. Finally Prevention is largely dependent on sanitation measures that may be difficult to implement, especially in developing countries [14].

11 DIAGNOSIS

As a result of the variety of clinical and often "flu like" symptoms, human leptospirosis is often undiagnosed or misdiagnosed as other illnesses with febrile syndromes (e.g. aseptic meningitis, influenza, hepatic disease, Hantavirus infections) [9], [12].

This is also the case in domestic animals, where most cases are difficult to diagnose clinically, due to non-specific clinical presentation or unapparent clinical signs with host-adapted serovars. Therefore, diagnosis of leptospirosis in both humans and animals cannot be made with confidence without laboratory confirmation [9]. Diagnosis of leptospirosis may be accomplished by direct detection of the organism or its components in body fluid or tissues, by isolation of leptospires in cultures, or by detection of specific antibodies and detecting the presence of leptospiral DNA [34], [9].

The collection of appropriate specimens and selection of tests for diagnosis depend upon the timing of collection and the duration of symptoms [12].

In humans, the first stage of the biphasic illness occurs before antibodies develop, and early cases must be diagnosed with assays that detect the organism, its antigens or nucleic acids. Leptospira may be found in the blood, cerebrospinal fluid, urine or tissue samples. In many cases, leptospirosis is diagnosed by serology, especially the MAT or ELISAs [35].

Diagnostic testing should be requested for patients in whom there is a high index of suspicion for leptospirosis, based either on signs and symptoms, or on occupational, recreational, or vocational exposure to animals or environments contaminated with animal urine [36].

11.1 MICROSCOPIC AGGLUTINATION TEST

The microscopic agglutination test (MAT) or Martin and Pettit test was developed almost one century ago at the Pasteur Institute [11]. It remains the reference method for serological diagnosis of leptospirosis for both humans and animals. Approximately 7 to 10 days after the onset of symptoms, antibodies can be detected by the MAT [9]. In the microscopic agglutination test (MAT), patients' sera are reacted with live antigen suspensions of leptospiral serovars. After incubation, the serum/antigen mixtures are examined microscopically for agglutination and the titers are determined. A serum is considered as positive, at a given dilution and for the tested antigen, if at least 50% of leptospires are agglutinated compared to a control antigen without serum. The MAT can be a complex test to control, perform, and interpret . Interpretation of the MAT is complicated by the high degree of cross-reaction that occurs between different serogroups, especially in acute-phase samples. [21], [12, [11].

The range of antigens used should include serovars representative of all serogroups. and all locally common serovars. The repeated weekly subculture of large numbers of strains presents hazards for laboratory workers, and laboratory-acquired infections have been reported [12].

At the Leptospirosis National Reference Center (French acronym CNRL) (Pasteur Institute), 24 strains are used (including non-pathogenic strain Leptospira biflexa strain Patoc 1 which has for particularity to cross-react with several antigens of pathogenic serogroups) to which may be added local strains from some over- seas regions for example. A smaller panel of antigens (according to French guidelines for biological procedures, the test should be performed with a minimum of nine antigens) may lead to non-detection of serogroups not presents in the panel [11].

11.2 OTHER SEROLOGICAL TESTS

Because of the complexity of the MAT, rapid screening tests for leptospiral antibodies in acute infection have been developed. Conventional serological methods such as enzyme-linked immunosorbent assay (Elisa) are widely used for the diagnosis of leptospirosis. Several IgM Elisa are available on the market, based on the detection of antibodies against a total extract of leptospires; usually the saprophytic strain L. biflexa, which shares several surface antigens with pathogenic strains. IgM antibodies become detectable during the first week of illness, allowing the diagnosis to be confirmed and treatment to be initiated while it is likely to be most effective. IgM detection has repeatedly been shown to be more sensitive than MAT when the first specimen is taken early in the acute phase of the illness [21,11]. In the event of symptoms in human contacts, infection can be assessed through the ELISA technique for initial screening [2]. A patient's serum may be positive 5 days after onset of symptoms but not usually before this period. In cases where antibiotic treatment has been initiated this period may be increased [3].

11.3 CULTURE

Requires special media. Leptospires can be isolated from whole blood (within 7 days of onset), cerebrospinal fluid (CSF) during the acute illness (4-10 days from onset), and from urine (after the 7th day and only if inoculated into special media within 2 hours of voiding). Clinical or autopsy specimens (e.g., punch biopsy of kidney) should be submitted fresh or frozen [36].

Venous blood is collected by means of an aseptic technique and ideally inoculated at the bedside into blood culture bottles containing culture medium for Leptospira. Small inocula consisting of a few drops of blood are inoculated into several tubes, each containing 5 ml of a suitable medium. Large inocula will inhibit the growth of leptospires. Cultures should be incubated at 30 °C and checked regularly for a period of 4–6 months[3].

For urine samples fresh midstream urine is collected and inoculated immediately. One drop of undiluted urine is inoculated into the first tube containing 5 ml of culture medium. Alternatively, urine samples may be centrifuged and the pellet resuspended in medium 30 min at 1600 g or 1 min at 10 000 g), after which 10-fold serial dilutions are made immediately in 1 or 2 additional tubes. Culture is carried out as for blood [3].

Incubation for up to 13 weeks at 30 ^{O}C with weekly examination by dark field microscopy (DFM) is necessary before cultures can be discarded as negative [6]. Leptospira spp. stain poorly with the Gram stain, and are not observed by microscopy unless special stains or methods are employed. Silver staining or immunogold-silver staining is sometimes useful as an adjunct technique. Dark field microscopy can also be used to detect Leptospira; however, this technique is non-specific and not very sensitive [1]. For this reason, culture is not considered useful as a routine test for diagnosis of individual patients, but remains important for epidemiological purposes and definitive [6,1].

11.4 POLYMERASE CHAIN REACTION (PCR)

This test has been developed for the rapid detection of Leptospira DNA. Leptospiral DNA has been amplified from serum, urine, aqueous humor, CSF, and a number of organs post mortem.

The PCR assays are more sensitive and have a higher specificity than conventional methods such as culture and dark-field microscopy. PCR analyses of blood samples are useful within the first 10-14 days of the disease. PCR on urine sample can differentiate the carrier or shedding state while serological tests can only detect antibody regardless of infection status at the time. A limitation of PCR-based diagnosis of leptospirosis is the current inability of PCR assays to identify the infecting serovar. While this is not significant for individual patient management [18,21].

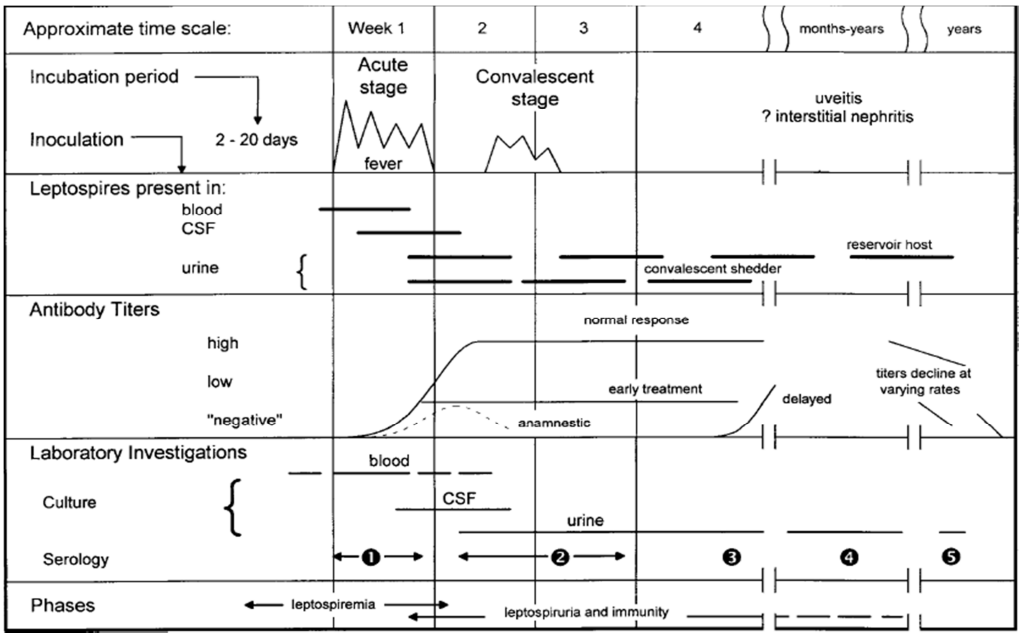

Fig. 3. Schematic representation of the biphasic nature of leptospirosis and relevant diagnostic investigations at different stages of disease. For serology specimens 1 and 2 are acute-phase specimens, 3 is a convalescent-phase specimen which may facilitate detection of a delayed immune response, 4 and 5 are follow-up specimens which can provide epidemiological information, such as the presumptive infecting serogroup. This figure was from [12], [9].

11.5 DIAGNOSTIC CONCLUSIONS

Currently, no diagnostic technique is completely satisfactory. The MAT and PCR are the two techniques allowing to confirm the diagnosis of leptospirosis (culture requires a one month incu-bation and does not allow making a quick diagnosis), but the MAT is used only by a few reference laboratories and detects antileptospires antibodies late (furthermore, a second sampling is advised to confirm leptospirosis) and PCR on a blood sample is possible only for a few days during the first week of the dis-ease. Thus, there is an urgent need to develop new techniques for an easy to use and quick detection of antibodies or antigens at the acute stage of the disease [11].

12 TREATMENT

Antibiotic treatment is effective within 7 to 10 days after infection and should be given immediately on diagnosis or suspicion. The drug of choice is benzyl penicillin by injection in the doses of five million units per day for five days. Patients who are hypersensitive to penicillin can be given erythromycin 250 mg four times daily for five days. Doxycycline 100 mg twice daily for ten days is also recommended. Tetracyclines are also effective but contraindicated in patients with renal insufficiency, in children and pregnant women [37].

Injection of Hydrocortisone 100mg every 8 hourly is also given in severe cases. Doxycycline has been used as a chemo prophylactic agent for short time exposure, but it cannot be recommended for routine continuous use or for a long-term occupational exposure [38].

13 TAXONOMY AND CLASSIFICATION

Leptospires are spirochetes, a group of bacteria that diverged early in bacterial evolution. The family leptospiraceae includes two genera, Leptospira and Leptonema [14].

13.1 SEROLOGICAL CLASSIFICATION

Prior to 1989, the genus Leptospira was divided into two species, L. interrogans, comprising all pathogenic strains, and L. biflexa, containing the saprophytic strains isolated from the environment [39]. Both L. interrogans and L. biflexa are divided into numerous serovars [40]. Over 60 serovars of L. biflexa have been recorded [40]. Within the species L. interrogans over 200 serovars are recognized; additional serovars have been isolated but have yet to be validly published. Serovars that are antigenically related have traditionally been grouped into serogroups [41].

13.2 GENOTYPIC CLASSIFICATION

Based on genetic homology in DNA hybridization experiments, 15 genomic species have been described in the genus Leptospira [42].

Genetic characterization is possible in only a few research laboratories and reference serological reagents (polyclonal and monoclonal antibodies) capable of defining serovars are not readily available [28].

14 LEPTOSPIROSE IN MOROCCO

In Morocco this disease is little known [43]. It was raised for the first time by Melnotte and Farjot who reported 7 cases of hemorrhagic spirochaétaes ictero-in Fez in 1927, and it is between 1950 and 1962 the maximum work and research on leptospirosis were conducted under the direction of Dr. White with the participation of Mailloux and Kolochine Erber [44].

M.HARAJI et al. [45] found in El Jadida Maroc one patient, 22 years old, was admitted to Mohamed V hospital in El Jadida presenting clinical symptoms assimilable with leptospirosis.

Mohamed ANOUAR SADAT [46] demonstrated that poultry market workers of Casablanca were at substantial risk of exposition to leptospirosis because of bad hygienic conditions and presence of rodents in both their workplace and their place of residence.

Recently we found in another study 7 serums were positives by Elisa during 1-1-2014 to 30-6-2015 majority of them were from Sidi kacem region. [47]

15 VACCINATION

Prevention of leptospirosis without vaccination is quite difficult. Measures for occupational hygiene such as protective clothing and avoidance of splash from urine or water are often useful but hard to implement because they impede work or are unacceptable to both workers and employers. For example, it is not practicable to advise dwellers in tropical villages to avoid hazardous activities like contact with pigs and other livestock or dogs, and walking or working in wet conditions, including in soil or water, such as rice paddies, contaminated by the animals' urine [6].

One of the first reports of human leptospirosis immunization involved the vaccination of thousands of miners in Japan using a culture-derived L. interrogans serovar Icterohaemorrhagiae vaccine [21].

The goal of vaccination is not to prevent the infection, but to prevent the infectious cycle of the disease. Another goal of vaccination is prevention of infection in humans. If the animals spread less disease, it is less likely that humans are infected due to contact with infected animals. Vaccination before infection protects the animal against the infection, most important it protect the animal against bacteria in the kidney. The protection is not a 100%. When vaccination is performed during infection it gives a better protection against reinfection. Infected animals will not stop shedding after vaccination. The protection of the vaccine is 6 to 12 months [18]. Commercial Leptospira vaccines are available globally for cattle, pigs and dogs but vaccination has proved to be only partially effective, due in part to the serovar restricted nature of vaccine induced immunity and the potential presence of local serovars others than those included in the vaccines. A successful vaccination program requires continued epidemiological studies to assess the incidence of different Leptospira serovars in a given population [6].

In humans the information on human vaccines is limited. These are available only in certain countries, such as China, Cuba , France and Russia As in animals, these vaccines are largely serovar-specific and protect for a relatively short period. Boosting at regular intervals is necessary to maintain protective titres of antibodies. These vaccines are also focused on the local situation and do not cover the needs in other regions where other serovars are endemic. Like the animal vaccines, those for humans are composed of crude antigens consisting of leptospires killed by phenol or formaldehyde that give unwanted side effects [34].

16 CONCLUSION

Leptospirosis is a widespread and potentially fatal zoonosis, it is endemic in many tropical regions and causes large epidemics after heavy rainfall and flooding. Although many wild and domestic animals can serve as reservoir hosts, rat is the most important source infections. Individuals living in urban slum environments characterized by inadequate sanitation and poor housing are at high risk of rat exposure and leptospirosis. Mortality increases with age, particularly in patients older than 60 years of age. The test method selected varies depending on the samples available and the purpose of testing. The diagnostic assay(s) used should be carefully chosen depending upon the circumstances and purposes of investigation. An increasing variety of laboratory methods are being described for detection of bacteria and antibodies. In addition to classical methods such as culture, dark-field, microscopy and the microscopic agglutination test (MAT), a variety of polymerase chain reaction (PCR), indirect enzyme-linked immunosorbent assay (ELISA).

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