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Current Status of Leptospirosis: A Zoonotic Tropical Disease

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Abstract

Leptospirosis occurs all over the globe but is maximum in the tropics. Leptospirosis, a spirochaetal zoonotic infection, has been documented as a significant rising infectious disease in the last several years. This review deals with the concerns in the epidemiology, diagnosis and clinical management which tackle community health responses, and focuses the advancement completed towards considerate the Leptospira genome, biology and pathogenesis. Although numerous wild and household animals can offer out as reservoir hosts, the brown rat (*Rattus norvegicus*) is the chief basis of human infections. Persons residing in city slum environments described by insufficient hygiene and poor shelter are at elevated risk of rat contact and leptospirosis. Antimicrobial treatment is specified for the severe leptospirosis, however its use is notorious for the mild form of leptospirosis. In a so-called outbreak, efforts to diagnose leptospirosis must be expectant to enable punctual treatment. For outbreaks in distant or areas with deprived access, limited utilization of screening tests to identify antibody is helpful. When an outbreak of leptospirosis is suspected or recognized, and if it has been promising to identify the attendant concerned, the source must be identified and appropriate environmental measures implemented, with public information to community at danger (including clinicians as well as health care employees and health authorities).

Keywords: Leptospirosis; Zoonotic disease; Diagnosis; Prevention and treatment.

Introduction

Leptospirosis is a global zoonosis originated by contamination with pathogenic Leptospira species, concerning almost all mammals [1,2]. Epidemic accounting for fatality worldwide emphasize the consequence of leptospirosis as a re-emerging, so far an ignored infectious disease. It is predictable that about half a million severe cases arise yearly around the world with a usual case fatality rate of 10% [3]. It is a zoonotic disease caused by spirochetes of the genus Leptospira. Disease frequently consequences when water or soil polluted with the urine of a contaminated animal appears in contact through human skin or mucous membranes. Quantifiable symptoms of leptospirosis can differ from a self-limited flu similar to febrile sickness to a fatal illness (Weil's disease) exemplified via jaundice, hemorrhage, renal failure, severe pulmonary hemorrhage and ARDS [4]. It is roughly more than hundred years since Weil, Professor of Medicine at Heidelberg (1886) whose name has been specified to the disease in humans first explained this disease, which is originated by *Leptospira interrogans*, serovar *icterohaemorrhagiae* or *copenhageni* [5]. Leptospires had been observed at that time, but were not cultured and were named *Spirocheta interrogans* by Stimson near the beginning of 1907, in silver stained preparations of liver as of a patient supposed to have died of yellow fever, the viral genesis of which were then unrecognized. The patient actually had Weil's disease [6]. It's contagious nature and microbial source were confirmed separately, first in Japan by Inada et al. *(Spirochaeta*

icterohaemorrhagiae) in 1916 [7], and almost immediately after in Germany (*Spirochaeta icterogenes*) by Uhlenhuth and Fromme [8]. Both groups isolated, cultivated and illustrated pathogenic Leptospires. Later, a saprophytic leptospira found in fresh water was illustrated in 1914; it was named *Spirochaeta biflexa*. Noguchi proposed the name 'Leptospira' (thin spirals) in 1918, subsequent detailed microscopical and cultural observations [9]. In the 15 years or so, from discovery until the 1930s, several of the important serovars prevalent all over the world, and their host sources were exposed [10]. During the 1920s to 1950s, the milder appearance of leptospirosis, the numerous linked but dissimilar serotypes and occupational relationships were explicated in Japan, Indonesia and Germany. Electron microscopy discovered much of the detail of the structure during the 1960s and 1970s [9]. Yanagawa and Faine (1966) demonstrated that Leptospires were analogous to other bacteria in structure and to characteristic antigens are related by structural elements [11]. Leptospirosis is basically water borne infection, as numerous outbreaks of disease have been confirmed in rainy season. There emerges to be straight relationship between the amount of rainfall and occurrence of disease [12]. The main transmission means are through injured skin and by means of long periods of contact to contaminated water or soil [13]. Leptospires can stay alive for weeks or months in the surroundings under favorable circumstances, such as temperatures of 28°C to 32°C and a neutral or slightly alkaline pH [2]. Leptospires are spirochetes that are transmitted among animals by straight or indirect contact. Exposure to contaminated urine, ingestion of infected tissues, and bite wounds are possibly the most frequent ways of direct transmission in the dog. Leptospira can be sporadically detected in the urine of recovered dogs for months to years following infection. Contaminated water sources, food or bedding are reasons of indirect transmission [14]. In human leptospirosis, it was considered formerly that diverse clinical syndromes were allied with definite serogroups, particularly serogroup Ictero haemorrhagiae, but this has now been disproved [4]. In contrast, it has been suggested recently that an exact clone in Leptospira interrogans serovar Copenhageni was connected to severe pulmonary haemorrhage syndrome [15]. A specific clone [a genotype defined by multi locus sequence typing (MLST)] of L. interrogans serovar Autumnalis was linked with outbreaks of leptospirosis in Thailand [16]. A serological study specified that members of sero group Pomona caused additional severe renal disease and were related with a inferior outcome in dogs than disease caused by serogroup Autumnal is or Grippotyphosa however, the differences in the virulence of different Leptospira genotypes in dogs remain unknown [17].

Etiology

Leptospirosis occurs universally, except in polar segments. It is believed to be the most widespread zoonosis in the world [18]. The occurrence is considerably higher in warm climate countries than in temperate regions, this is owing to mainly to longer survival of leptospires in the atmosphere in warm, humid conditions [4]. Infected animals might have leptospires persistently colonizing the proximal renal tubules and excrete the organism sporadically for months or years, or still for life span [2]. Disease is continued by chronic carrier hosts that excrete the organism into the surroundings, and infection in man consequences from direct contact with infected animals or indirect contact with a contaminated atmosphere [19]. Although excretion of leptospires in human urine for weeks or months, humans are not observed as a source of transmission [1]. Leptospires are spirochetes, about 0.1 mm in diameter by 6–20 mm in length and contain both saprophytic and pathogenic species include the genus Leptospira, which belongs to the family Leptospiraceae, order Spirochaetale [2]. At the 2007 summit of the Subcommittee on the Taxonomy of Leptospiraceae held in Quito, Ecuador, it was determined to provide the class of species to the formerly explained genomo species 1, 3, 4 and 5, resulting in a family comprising 13 pathogenic Leptospira species: *L. alexanderi*, *L. alstonii* (genomospecies 1), *L. borgpetersenii*, *L. inadai*, *L. interrogans, L. fainei, L. kirschneri, L. licerasiae, L. noguchi, L. santarosai, L. terpstrae* (genomospecies 3), *L. weilii, L. wolffii*, by more than 260 serovars. It is expected that additional new species exist. Saprophytic species of Leptospira include *L. biflexa, L. meyeri, L. yanagawae* (genomospecies 5), *L. kmetyi, L. vanthielii* (genomospecies 4), and *L. wolbachii*, and contain more than 60 serovars. On the other hand, the serovar categorization of Leptospira is based on the expression of the surface-exposed epitopes in a mosaic of the lipopolysaccharide (LPS) antigens, because the specificity of epitopes depends on their sugar composition with orientation. Leptospires contain distinctive hooked ends. Two periplasmic flagella by polar insertions are located in the periplasmic space and are responsible for motility; the FlaA and FlaB proteins comprise the flagellar sheath and core respectively. Electron microscopy demonstrated a flab mutant to be deficient in endo flagella and non-motile [20]. Leptospires are thin, obligate aerobe, fine spiral shaped organisms with hooked ends have two or additional axial filaments that are dependable for the action of the spirochete, and are envisaged under dark field microscopy. The epidemiology of most serovars is weakly studied except certain serovars have been connected powerfully to specific animal reservoirs [21]. A number of the serotypes are present worldwide, as others are restricted to certain areas. Usually known serovars are *L. interrogans serovars* Pomona (swine), *L. interrogans* Bratislava (swine*), L. interrogans* Canicola (dogs), *L.interrogans* Bovis (cattle), *L. interrogans* Autumnalis (raccoons), and Icterohemorrhagiae and Copenhageni (rats) [22]. The organism is sensitive to ordinary disinfectants and antiseptic, and simply killed at 60°C in 10 seconds. The case fatality range from 3 to over 50% [23].

Clinical Spectrum and Laboratory Diagnosis

Leptospires cross the threshold of the body through tiny cuts or abrasions, via mucous membranes such as the conjunctiva or through damp skin. They move in the blood stream, with the bacteremic phase lasting for up to 7 days.

After the numbers of leptospires in the blood and tissues attain a critical level, lesions owing to the action of undefined leptospiral toxin(s) or toxic cellular mechanisms and consequent symptoms appear. The primary cut is damage to the endothelium of minute blood vessels leading to localized ischemia in organs, ensuing in renal tubular necrosis, hepatocellular and pulmonary damage, meningitis, myositis and placentitis. Hemorrhages take place in harsh cases as do jaundice, and frequently, platelet deficiency. There is typically a mild granulocytosis and splenomegaly. Once circulating antibodies emerge, leptospires are removed from the circulation and tissues by opsonophagocytosis. Tissue damage, even though it is severe, may be reversible and followed by absolute repair (e.g. kidney, liver) although long lasting damage (e.g. myocarditis) may be a complication and may directed to scarring, well recognized in the kidneys of pigs and dogs, where it may be observed macroscopically as ''white spots''[24].

Laboratory Diagnosis

Specimen collection

A number of blood tubes should be collected at the early phase of the disease: normal blood culture bottle or tube; Non additive or gel separator tubes for chemistry and serology; and EDTA tube for blood count. For blood culture, blood with heparin to stop clotting is recommended but perfectly blood is inoculated directly into blood culture bottles including culture medium for leptospires [25-27].

Microscopic demonstration

Leptospires cannot be examined under the ordinary light microscope but by dark field microscopy as thin, coiled, and rapidly moving microorganisms. Compassion of dark field microscopy is approximately 10^7 leptospires/L. Through examination of blood and urine has both low sensitivity and specificity, it is issue to misunderstanding of fibrin or protein threads, afterward is not recommended as a regular procedure. Leptospires are not stained by conventional Gram staining. Existing staining methods to augment the sensitivity of direct examination are: immunofluorescence, immunoperoxidase, silver staining, Warthin-Starry staining, immunohistochemistry, and *in situ* hybridization. All of these suffer from the same disadvantage as dark field microscopy: an elevated risk of false-positive and false-negative results.

Isolation of leptospires

Samples for culture should be collected earlier to the administration of antibiotics. Blood, cerebrospinal fluid and dialysate should be cultured in the first 10 days of the illness, and urine from the second week of the illness. Several specific media were explained by Fletcher et al. The majority used medium is based on the oleic acid-albumin Ellinghausen-McCullough-Johnson-Harris (EMJH) medium (Becton Dickinson and Company, Difco_) and is accessible commercially. Samples should be stored and transported at ambient temperatures. Survival of leptospires in human urine is restricted so urine should be processed instantly. Cultures are incubated in the dark at 28-30°C and observed weekly by dark field microscopy for up to 13 weeks prior to being discarded [28].

Antigen detection

Different antigen detection trials have been developed but none of them is sensitive enough to be characteristically used [29].

Antibody detection

The MAT (microagglutination test), that is the serological reference test, was first explained in 1918 by Martin and Pettit. Live antigens representing diverse serogroups are reacted with serum samples and the agglutination is examined by darkfield microscopy. Panels of live leptospires belonging to different serovars have to be preserved in the laboratory [30].

Evaluation of rapid screening tests

Rapid tests are simple to employ and can be performed by persons without special technical training. Various of them can be performed on whole blood and can be stored for prolonged periods at ambient temperatures, and standard laboratory equipment is not necessary [31]. Eight rapid tests (IHA, 2 IgM dipstick assay; indirect fluorescent antibody, 3 ELISA IgM, LA) have been evaluated in Hawaii and the authors concluded that all tests were insensible for diagnosis within the first week of the ailment as it is during this time that significant therapeutic decisions are likely to be completed [32]. The low sensitivity of these tests at the acute phase of the illness is not linked to the rapid test set-up but is due to the fact that the tests detect IgM antibodies. [28].

Molecular diagnosis

The need for quick diagnostics at the occasion of admission has led to the growth of numerous PCR assays. Their benefit lies in the skill to obtain a definitive diagnosis during the acute phase of the illness prior to antibodies are measurable, while treatment may be successful. PCR detects DNA in blood in the first 5-10 days after the start of the disease and up to the 15th day.

Epidemiology

Leptospirosis is a clinical entity; it is frequently under diagnosed and under reported. Recent information on worldwide human leptospirosis differs but shows that approximately 1 million severe cases occur per year, with a mean case-fatality rate of nearly 10% [33]. The majority of cases occur in men. Estimated 100-200 cases are recognized yearly in the United States, with about 50% of cases in Hawaii, Indonesia as a nation with high leptospirosis cases, third rank of death rate in the world. Indonesia Ministry of Health gave details that there were 641 human cases in 2013 with case-fatality rate was 9.36%. Outbreak of leptospirosis is not well understood. Epidemic may effect from exposure to flood waters contaminated by urine from infected animal, as has been made clear from several countries. Recreational disclosure and domestic animal contact are well-known sources of leptospirosis. Occupational exposure may be accounts for leptospirosis [34-36].

Treatment

In canine leptospirosis, renal and liver failures are potentially reversible and should be treated as early on and insistently as possible. The affected dogs are treated symptomatically by means of antiemetics and gastric protectants, and particular alertness is paid to sufficient urine production after the animals have been correctly rehydrated. The placement of a sterile urinary catheter can be supportive in assessing urine production as well as containing potentially infective waste. Urine production <2 ml/kg/hr in a sufficiently hydrated dog shows oliguria and must be treated insistently. In animals that are not liquid overloaded, mannitol is frequently considered as an alternative treatment. It is primarily given as a bolus, (0.5 g/kg over 30-60 min) and then followed as a steady rate infusion (1-2 mg/kg/min) if urine production retorts appropriately. Otherwise, furosemide can also be administered. It is primarily given as a bolus (2-4 mg/kg) and then followed as a constant rate infusion (0.25-1 mg/kg/hour) if urine production raises suitably. Urine production should be followed intimately and over-hydration of the patient avoided. Antibiotic treatment is usually given in 2 phases: ampicillin or amoxicillin can be administered parenterally (20-25 mg/kg i.v. TID) during the initial, critical phase. It is noteworthy to note that the kidneys clear these drugs and blood concentrations can develop into inappropriately high in patients with renal dysfunction [14]. Leptospirosis in all its appearances is amenable to treatment with antibiotics. Leptospires are susceptible in laboratory tests to all clinically useful antibiotics, excluding chloramphenicol and rifampicin. Leptospires resistance in clinical use has not been reported, although breakdown of treatment have hardly ever been suggested. The antibiotics that are majority typically recommended are penicillin, at high doses, unless the patient is hypersensitive to penicillin, in which case erythromycin is used. Tetracyclines are used apart from they have disadvantages and are contraindicated for group with renal insufficiency, for children and for pregnant women. Doxycycline is indicated for treatment and short-term chemoprophylaxis. Penicillin should be administered as early as possible, throughout the leptospiraemic phase, parenterally in extremely ill patients. No antibiotic can overturn the destructive effects of leptospirosis in tissues and organs, but penicillin was found to have useful effects, reducing mortality and extent of illness in severe leptospirosis, when given intravenously, still at a late stage. Antibiotic treatment may be attended by a Jarish-Herxheimer reaction (a transient increase in fever and severity of symptoms immediately following treatment), which does not contraindicate administration of antibiotics [37]. Kidney failure is the commonest reason of death; if there are signs that this is growing, peritoneal dialysis or the use of an artificial kidney should not be postponed [5]. Antibiotic treatment is successful within 7 to 10 days after infection and should be given instantly on diagnosis or doubt. The drug of choice is benzyl penicillin by parenteral route in the doses of five million units per day for five days. Patients those 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 as well recommended. Tetracyclines are also efficient but contraindicated in patients with renal insufficiency, in kids and pregnant women [38]. Injection of Hydrocortisone 100 mg after each 8 hours is also given in severe cases. Doxycycline has been employed as a chemo prophylactic agent for short time exposure, but it cannot be intended for routine continuous use or for a long-term job-related exposure [39].

Prevention and Control

Prevention of leptospirosis without vaccination is quite difficult [24].

Individual protection

Sanitary methods such as prevention of direct and indirect human contact with animal urine are suggested as preventive measures. Workers should be concerned against direct contact with contaminated water or mud and should be advised to utilize rubber shoes and gloves. In case of any cuts or abrasion on the lower ends of the body, the worker should apply an antibacterial ointment e.g. betadine.

Health education

The major defensive measure for leptospirosis is to create consciousness about the disease and its prevention. Rodent control has to be customary beyond doubt that rodents are the major reservoirs of bacterium. Therefore controlling this reservoir genus with correct strategy planning and management planning will reduce the occurrence of the disease in the affected areas. The strategic planning should cover the following:

1. Recognizing the reservoir species of affected area.
2. Defining areas for anti rodent activities.
3. Ending of activities in pre monsoon months.
4. Accepting appropriate skill for anti rodent operations. This comprises correct inputs and suitable application technology.
5. Ability building
6. Making awareness in general community and community contribution [24,40].

Conclusion

Leptospirosis is a global fatal zoonosis it is endemic in a lot of tropical area and causes large epidemics after heavy rainfall and flooding originated by infection with pathogenic Leptospira species, affecting almost all mammals. Although several wild and domestic animals can serve as reservoir hosts, rat is the majority important source of infections. Persons living in urban slum surroundings characterized by insufficient hygiene and poor housing are at high danger of rat exposure and leptospirosis. Deaths increases with age, mainly in patients older than 60 years of age. The test technique selected varies depending on the samples existing

and the reason of testing. The diagnostic assay(s) employed should be cautiously selected depending upon the circumstances and purposes of investigation. A rising variety of laboratory methods are being explained for detection of bacteria and antibodies. In addition to traditional methods such as culture, dark-field, microscopy and the microscopic agglutination test (MAT), a range of polymerase chain reaction (PCR), indirect enzyme-linked immunosorbent assay (ELISA). Human to-human transmission takes place only very rarely. Leptospires are bacteria which can also be pathogenic (i.e. having the probability to originate disease in animals and humans) or saprophytic (i.e. free living and generally believed not to cause disease). Pathogenic leptospires are preserved in nature in the renal tubules and genital tracts of certain animals. Leptospirosis occurs globally but is the majority common in tropical and subtropical areas with high rainfall. The disease is found mostly wherever humans come into contact with the urine of infected animals or a urine polluted surroundings. Moisture is a significant factor of the survival of the leptospires in the environment. Additional modes of transmission of infection, such as handling infected animal tissues and ingestion of infected food and water, are also possible. It is usually supposed that serovar-specific antibodies are protective and that a patient is resistant to reinfection with the same serovar as extended as the concentration (titre) of specific antibodies is high enough. Antibodies aggravated by an infection with a particular serovar do not essentially defend against infection with other serovars. Treatment with effective antibiotics should be started as soon as the diagnosis of leptospirosis is suspected and if possible before the fifth day after the beginning of illness. The advantage of antibiotics after the fifth day of the disease is controversial. Though, the majority clinicians treat with antibiotics in spite of the date of onset of the illness. Clinicians should not at all wait for the results of laboratory tests before preliminary treatment with antibiotics. Since serological tests do not become positive until about a week after the beginning of illness, and cultures may not become positive for several weeks. In severe cases, admission to a hospital is required. Aggressive supportive care with strict consideration to fluid and electrolyte balance is necessary. Peritoneal or haemodialysis is indicated in renal failure. Automatic ventilation is indicated for lung hemorrhagic manifestation. Tremendous supportive care and dialysis have lessened the mortality due to this illness in current years.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Bharti AR, Nally JE, Ricald JN. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis*. 2003; 3(12): 757-771. doi: 10.1016/S1473-3099(03)00830-2
2. Faine S, Adler B, Bolin C, Perolat P. Leptospira and Leptospirosis. 2nd edition. Melbourne: *Medi Sci*. 1999.
3. Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clin MicrobiolInfect*.2011;17(4):494-501.doi:10.1111/j.1469-0691.2011.03474.x.
4. Levett PN. Leptospirosis. *Clin Microbiology Rev*. 2001; 14(2): 296–326. doi: 10.1128/CMR.14.2.296-326.2001
5. Edward A, Staughton H. Leptospirosis. In: Topley and Wilson's Principles of Bacteriology, Virology and Immunity. 8th edition. 1991; 3: 619.
6. Stimson AM. Note on an organism found in yellow fever tissue. *Public Health Rep*. 1907; 22: 541-581. doi: 10.2307/4559008
7. Inada R, Ido Y, Hoki R, Kaneko R, Ito H. The etiology mode of infection and specific therapy of Weil's disease. *J Exp Med*. 1916; 23(3): 377-402. PMID: 19867994
8. Uhlenhuth P, Formme W. In: Topley and Wilson's Principles of Bacteriology, Virology and Immunity. 1916; 8(2): 617.
9. Noguchi H. The spirochetes, in the newer knowledge of bacteriology and immunology. Jordan EO, Falk IS (eds). University of Chicago Press, Chicago. 1928; 452-497.
10. Kmety E, Dikken H. Classification of the species *Leptospira interrogans* and the history of its serovars. University Press Groningen, Groningen. 1993.
11. Yanagawa R, Faine S. Morphological and serological analysis of Leptospiral structure. *Nature*. 1996; 11: 823-826. doi: 10.1038/211823a0
12. Pal M. Leptospirosis: A contemporary zoonosis. The Veterinarian. 1996; 20: 11-12.
13. Faine S. Leptospira and leptospirosis. 2nd edition. CRC Press Inc. 1994.
14. Gething M, Jones B, World Small Animal Veterinary Association. Federation of European Companion Animal Veterinary Associations, & Veterinary Ireland Companion Animal Society. Programme and scientific proceedings: 33rd annual World Small Animal Veterinary Association Congress, Dublin, Ireland. 2008: 320-322.
15. Gouveia EL, Metcalfe J, Carvalho ALF, et al. Leptospirosis-associated severe pulmonary hemorrhagic syndrome, Salvador, Brazil. *Emerg Infect Dis*. 2008; 14(3): 505–508. doi: 10.3201/eid1403.071064
16. Thaipadungpanit J, Wuthiekanun V, Chierakul W, et al. A dominant clone of *Leptospira interrogans* associated with an outbreak of human leptospirosis in Thailand. *PLoS Neg Trop Dis*. 2007; 31: 56. doi: 10.1371/journal.pntd.0000056
17. Goldstein RE, Lin RC, Langston CE, Scrivani PV, Erb HN, Barr SC. Influence of infecting serogroup on clinical features of leptospirosis in dogs. *J Vet Intern Med*. 2006; 20(3): 489-494. doi: 10.1111/j.1939-1676.2006.tb02886.x
18. Picardeau M. Diagnosis and epidemiology of leptospirosis. *Med Mal Infect*. 2013; 43(1): 1-9. doi: 10.1016/j.medmal.2012.11.005
19. Boonsilp S, Thaipadungpanit J, Amornchai P, Wuthiekanun V, Chierakul W, Limmathurotsakul D. Molecular detection and speciation of pathogenic Leptospira spp. in blood from patients with culture-negative leptospirosis. *BMC Infect Dis*. 2011; 11: 338. doi: 10.1186/1471-2334-11-338
20. Picardeau M, Brenot A, Saint Girons IS. First evidence for gene replacement in Leptospira spp. Inactivation of *L. biflexa* fla B results in non-motile mutants deficient in endoflagella. *Molecular Microbiology*. 2001; 40(1): 189-199. doi: 10.1046/j.1365-2958.2001.02374.x
21. Slack A. Leptospirosis. *Aust Fam Physician*. 2010; 39(7): 495-498. PMID 20628664.
22. Heymann D. Control of communicable diseases manual. American Public Health Association. KIT Biomedical Research. 2004; 306-309.
23. WHO. Human Leptospirosis: Guidance for diagnosis, surveillance and control. World Health Organization and International Leptospirosis Society, WHO library Cataloguing-in Publication Data, Malta. 2003.
24. Adler B, de la Pena Moctezuma A. Leptospira and Leptospirosis. *Veterinary Microbiology*. 2010; 140: 287-296. doi: 10.1016/j.vetmic.2009.03.012
25. Agampodi SB, Matthias MA, Moreno AC, Vinetz JM. Utility of quantitative polymerase chain reaction in leptospirosis diagnosis: association of level of leptospiremia and clinical manifestations in Sri Lanka. *Clin Infect Dis*. 2012; 54(9): 1249-1255. doi: 10.1093/cid/cis035
26. Stoddard RA, Gee JE, Wilkins PP, McCaustland K, Hoffmaster AR. Detection of pathogenic Leptospira spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. *Diagn Microbiol Infect Dis*. 2009; 64(3): 247-255. 15. doi: 10.1016/j.diagmicrobio.2009.03.014
27. Bourhy P, Bremont S, Zinini F, Giry C, Picardeau M. Comparison of real-

time PCR assays for detection of pathogenic Leptospira spp. in blood and identification of variations in target sequences. *J Clin Microbiol*. 2011; 49: 2154-2160. doi: 10.1128/JCM.02452-10

28. Musso D, La Scola B. Laboratory diagnosis of leptospirosis: A challenge. *J Microbiol Immunol Infect*. 2013; 46(4): 245-252. doi: 10.1016/j.jmii.2013.03.001

29. Champagne MJ, Higgins R, Fairbrother JM, Dubreuil D. Detection and characterization of leptospiral antigens using a biotin/avidin double-antibody sandwich enzyme-linked immunosorbent assay and immunoblot. *Can J Vet Res*. 1991; 55(3): 239-245.

30. Chou YL, Chen CS, Liu CC. Leptospirosis in Taiwan, 2001-2006. *Emerg Infect Dis*. 2008; 14(5): 856-857. doi: 10.3201/eid1405.070940

31. World Health Organization, Pan American health organization. Commercially available simple/rapid test kits for diagnosis of emerging and re-emerging infections in the region of the Americas. Geneva: WHO. 2013.

32. Effler PV, Bogard AK, Domen HY, Katz AR, Higa HY, Sasaki DM. Evaluation of eight rapid screening tests for acute leptospirosis in Hawaii. *J Clin Microbiol*. 2002; 40: 1464-1469. **doi: 10.1128/JCM.40.4.1464-1469.2002**

33. Hartskeerl RA, Wagenaar JF. Leptospirosis. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson JL, Loscalzo J (eds). Harrison's principles of internal medicine. 2015; 19: 1140-1145.

34. Scoot GM, Coleman TJ. (209) Leptospirosis. In: Farrar J, Hotez P, Junghanss T, Kang G, Lalloo D, White NJ (eds). Manson's tropical diseases. 23rd edition. 2014.

35. Magill AJ, Ryan ET. Leptospirosis Hunter's tropical medicine and emerging infectious diseases 9th edition, Saunders Elsevier: 597-601. doi: 10.1016/C2009-0-51934-4

36. Indonesian Public Health Portal. Epidemiologic Leptospirosis. 2016.

37. Faine S. Leptospirosis. In: Topley and Wilson's Microbiology and Microbial infections. 9th Edition. 1998; 3: 849-869.

38. Spichler A, Moock M, Chapola EG, Vinetz J. Weil's disease: an unusually fulminant presentation characterized by pulmonary hemorrhage and shock. *BJID*. 2005; 9(4): 336-340.

39. Gonsalez CR, Casseb J, Monteiro FG, Paula-Neto JB, Fernandez RB, Silva MV. Use of doxycycline for leptospirosis after high-risk exposure in Sao Paulo, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*. 1998; 40(1): 59-61.

40. Haake DA, Levett PN. Leptospirosis in Humans. *Curr Top Microbiol Immunol*. 2015; 387: 65-97. doi: 10.1007/978-3-662-45059-8_5