

Leptospirosis: recent incidents and available diagnostics – a review

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SUMMARY

Objective: The aim of this article was to review published research articles on leptospirosis, in particular the recent incidence of leptospirosis in Malaysia and the currently available diagnostic methods for the detection of leptospirosis.

Methods: PubMed, Google Scholar and Google Search databases were searched using the key words *Leptospira* and leptospirosis. A total of seventy-six references were reviewed including sixty-seven research articles, three annual reports from Ministry of Health and six online newspaper articles. This review includes the following five sub-headings: introduction, leptospirosis transmission, leptospirosis incidents, laboratory diagnosis of leptospirosis and treatment and prevention of leptospirosis.

Results: An increase in incidents of leptospirosis cases has been seen in recent years in Malaysia. The recent floods have contributed to the rise in the number of reported cases. Current diagnostic approaches such as dark field microscopy, microscopic agglutination test (MAT), Polymerase chain reaction and serological tests are inadequate as the organism is a slow grower.

Conclusion: There is an urgent need to develop newer techniques for rapid detection of leptospirosis. The combination of PCR and ELISA are suggested for rapid and accurate diagnosis of leptospirosis. Studies on the mechanism of pathogenesis of *Leptospira* are needed for the development of vaccines that are safe for human use.

KEY WORDS:

Leptospira, *Leptospirosis*, *diagnosis*, *treatment*, *prevention*

INTRODUCTION

Leptospirosis is a zoonotic disease occurs worldwide. It is endemic in tropical and subtropical countries in South-East Asia.¹ An increase in incidents has been seen in recent years due to flooding and outdoor recreational activities.² The World Health Organization (WHO) states that the global prevalence of leptospirosis is over one million severe human cases per year.^{3,4} Accurate clinical diagnosis can be difficult without laboratory confirmation and the disease burden is generally under-reported. WHO has formed the Leptospirosis Burden Epidemiology Reference Group (LERG) to establish correct estimations of the disease burden for better adequate intervention, control and prevention.⁴ The incubation period

for leptospirosis is between two days and four weeks and the illness begins abruptly with fever and other symptoms. Leptospirosis may occur in two phases. In the first phase, the symptoms which can be observed are fever, chills, severe head ache, nausea, vomiting, myalgia and diarrhoea. Many of these symptoms can be mistaken for other diseases. The patient usually recovers for a time but become ill again. The second phase is called Weil's disease and is more severe and the symptoms include sepsis with multiple organ dysfunction, jaundice and haemorrhage which turn to circulatory shock and eventually death.⁵ The illness lasts from few days to three weeks and without treatment, recovery may take several months.⁶ In this review, the recent incidents in Malaysia and the available diagnostics for leptospirosis are discussed.

Leptospirosis is a water-borne disease mainly transmitted by rodents

Leptospire are spirochetes, which are thin, highly motile with an approximate size of 0.1µm in diameter and 6-20 µm in length with a distinctive hook or question-mark shape.⁷ The genus *Leptospira* can be divided into pathogenic and saprophytic strains, which belongs to the family Leptospiraceae and order Spirochaetales. Currently, there are 21 species, 25 serogroups and 250 serovars of pathogenic leptospire have been described.^{6,8} Leptospire are slow-growing obligate aerobes with an optimal growth temperature of 28°C-30°C.⁹ The most widely used media to culture leptospire is the Ellinghausen-McCulloch-Johnson-Harris (EMJH) medium which contains oleic acid, bovine serum albumin (BSA), and polysorbate (Tween)⁷. The genome size of *Leptospira* is approximately 3.9-4.6 Mb (megabase) depending on the species. Six genome sequences of *Leptospira* have been published including two isolates of *L. interrogans* (serovar Lai and Copenhageni), two isolates of *L. borgpetersenii* (serovar Hardjo) and two isolate of *L. biflexa* (serovar Patoc).^{7,10,11}

The disease occurs wherever humans come into contact with the urine of infected animals or a urine polluted environment.¹² The usual portal of entry of leptospire is through cuts or abrasions in the skin. Leptospire also invade the human body through nasal, oral and conjunctival mucous membranes when exposed to contaminated water for a prolonged period of time. Inhalation of the aerosol containing leptospire may cause infection of the respiratory tract. The transmission of leptospirosis from human to human is rare. However, leptospire can be transmitted through sexual intercourse and breastfeeding.^{12,13} Once the

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leptospire invade the human body, it enters the blood circulation and attacks the organs and tissues. The excretions of leptospire in urine of humans occur continuously for up to 11 months. The main carriers of pathogenic strains of leptospire are cattle, pigs, buffaloes, dogs and particularly rodents.⁹ They are called as natural maintenance host as they serve as the main reservoirs for pathogenic leptospire. Importation of animals also leads to the transmissions of leptospirosis to the domestic livestock, pet animals or native wildlife.¹⁴ The transmission of leptospire depends on many factors, including climatic condition, population density, and the degree of contact with the host.¹⁵ Occupational exposure is the risk factor for sewage workers, peasants, veterinarians, miners and rodent control workers due to high degree of contact with the animal host or contaminated water and soil.^{16,17}

Although the mechanism of pathogenesis remains unclear, several virulence factors have been implicated to the pathogenicity which includes LPS, haemolysins, outer membrane proteins (OMPs) and other surface protein.¹⁸ LPS of *Leptospira* resembles standard gram-negative LPS immunologically and chemically with a reduced biological activity in endotoxic assays.^{7,19,20} *Leptospira* haemolysins have been suggested to be phospholipases that causes holes in erythrocyte of sheep, calves and other mammalian cells.^{21,22} OMPs of *Leptospira* have been reported to induce immune response against the disease, particularly LipL32, a 32kDa lipoprotein. Antibodies to LipL32 serve as an important serologic marker during leptospirosis.²³⁻²⁷

Leptospirosis incidents shows an increasing trend in recent years in Malaysia

Adolf Weil in 1886 discovered and published the details of severe form of leptospirosis.²⁸ Inada and colleagues in 1916 demonstrated spirochetes in the liver tissue of a guinea pig injected with the blood of a patient suffering from infectious jaundice.^{29,30} Two groups of German physicians succeeded in transmitting leptospirosis to guinea pigs and they gave the names, *Spirochaeta nodosa* and *Spirochaeta icterogenes*.^{31,32} The nomenclature of *Leptospira* was introduced by Arthur Stimson when he isolated spirochete from kidney tissue sections of a patient thought to have died of yellow fever in 1907. This was followed by the discovery of a concentrated bacterium in the renal tubules with a question mark shape that gives the name *Spirocheta interrogans*.³³

Leptospirosis was first reported in Malaysia in 1925. A study conducted among the town service workers in Malaysia indicated a high seroprevalence among garbage collectors (27.4%), followed by town cleaners (23.8%), landscapers (13.8%) and lorry drivers (17.9%). High seroprevalence among garbage collectors was considered due to longer duration of exposure and close contact with the garbage that might be contaminated with *Leptospira* infected rat urine.¹ High seroprevalence was also noted among people living within the national park, Sabah which can be attributed to exposure to the wild animals.¹⁵

Since December 2010, Leptospirosis has been gazetted as a notifiable disease under Prevention and Control of Infectious

Disease Act 1988. According to Malaysia Ministry of Health, the number of Leptospirosis cases has increased dramatically in the past 5 years with 22,566 reported cases and 296 fatalities in the age group between 20 to 60 years. More than half of the cases reported were males. The highest number of reported cases and fatalities was in 2014 with 7806 cases and 92 fatalities. Of the 7806 cases reported, Selangor has the highest number with 1832 cases.³⁴⁻³⁷ Up to July 2015, the number of Leptospirosis reported cases in Malaysia was 5370 and 30 fatalities with high incidence in 5 states including Kelantan, Selangor, Sarawak, Kedah and Terengganu. As shown in Table I, only sporadic cases were observed in Sabah while from January 1 to January 18, 2015, a total of 647 suspected and 110 confirmed cases of leptospirosis were reported after the floods in Kelantan, Perak, Terengganu and Pahang which might be due to leaching of leptospire from the soil.

Laboratory diagnosis of leptospirosis

Diagnosis of leptospirosis is difficult due to the wide diversity of clinical symptoms which mimic regular symptoms of fever. The diagnostic methods include direct examination by dark field microscopy, rapid nucleic acid test by Polymerase Chain Reaction (PCR) and rapid antibody-based tests.⁴⁶ Use of combination of laboratory tests is recommended for accurate diagnosis.

Dark Field Microscopy (DFM) has low sensitivity

Approximately 10^2 to 10^6 leptospire/ml of blood is necessary for visualization by dark field microscopy which results in low sensitivity compared to other diagnostic methods.^{46,47} The sensitivity of DFM was shown to be 61% and 93.3% and the specificity was 60%.^{48,49} Although, this diagnostic method involves low cost, there is a risk of false positives due to the misinterpretation of fibrin or other protein threads and thus not recommended for diagnosis.

Rapid Nucleic Acid Diagnostic Tests are rapid and specific

Polymerase Chain Reaction (PCR) based method has gained popularity for diagnosis of leptospirosis. Conventional and nested PCR have been developed previously, for the detection of leptospire targeting specific genes, particularly *LipL32* or *secY* genes.⁴⁹ PCR-based method usually detects DNA in the blood sample in the first 5 to 10 days after the onset symptoms and up to day 15. The detection limits of PCR assay is 100-1000 bacteria per millilitre of blood or urine sample.^{50,51} The conventional PCR assay has been replaced by real-time quantitative PCR (RT-PCR) such as SYBR Green qPCR and TaqMan PCR which are much faster. The sensitivity and specificity of RT-PCR targeting *rss* gene was shown to be 56% and 90% respectively.⁵² While the sensitivity and specificity of RT-PCR targeting *lipL32* gene was reported to be 43-93% and 97-100% respectively.^{52,53} Multiplex assays have been developed for simultaneous detection and differentiation of pathogenic and nonpathogenic *Leptospira*.⁵⁴ PCR is sensitive, specific, and become positive early in disease and able to detect leptospire DNA in blood, urine, cerebrospinal fluid (CSF) and aqueous humour. However, this test appears to be genus specific and not serovar specific and requires highly skilled personnel to perform the test to prevent contamination and false positive results.⁶

Table I: Recent incidence of leptospirosis in Malaysia

S.No	Year	Location	Fatalities	Positive cases	Reference
1	2010	Hutan Lipur Lubuk Yu, Maran	6		Lim <i>et al.</i> 2011 ³⁸
2	2011	Eastern Pahang	8		Lim <i>et al.</i> 2011 ³⁸
3	2013 (Nov)	Putatan, Sabah	2		BorneoPost Online, 2013 ³⁹
4	2014 (April)	Sibu, Sarawak	6		Boon, 2013 ⁴⁰
5	2014 (October)	Labuan Matriculation College	1		ESSCOM TIMES, 2014 ⁴¹
6	2014 (Nov)	Labuan Matriculation College		2	BorneoPost Online. 2014 ⁴²
7	2015 (Jan 1-15)	Kelantan		458 (49)*	Abdullah, 2015 ⁴³
		Perak		113 (21)*	
		Terengganu		39 (26)*	
		Pahang		37 (14)*	
8	2015 (June)	Jempol, Pahang	1	14	Pei Ying, 2015 ⁴⁴
9	2015 (June)	Sibu, Sarawak	1	10	Chua, 2015 ⁴⁵

*(Figures in parenthesis: confirmed cases)

Table II: Laboratory diagnostic methods for leptospirosis

Diagnosis method		Sensitivity	Specificity	Reference
Microbiology	Dark Field Microscopy (DFM)	61%	60%	Sharma and Kalawat, 2008 ⁴⁸
		93%		Chandrasekaran and Gomathi, 2004 ⁴⁹
Serology	Microscopic Agglutination Test (MAT)			
	1-5 days	30%	97%	Cumberland <i>et al.</i> 1999 ⁵⁵
	5-14 days	63%		Cumberland <i>et al.</i> 1999 ⁵⁵
	Convalescent	76%	86.7%	Cumberland <i>et al.</i> 1999 ⁵⁵
		91.4%		Eugene <i>et al.</i> 2015 ⁵⁶
	Enzyme-linked Immunosorbent Assay (ELISA)			
	IgM	86.5%	97%	Silva <i>et al.</i> , 1995 ⁵⁸
	rLIPL32	96.4%	90.4%	Vedhagiri <i>et al.</i> 2013 ⁶⁴
	Immunofluorescence (IF)			
		45%	96.8%	Limmathurotsakul <i>et al.</i> 2012 ⁶⁵
	91.9%	100%	Kemapunmanus <i>et al.</i> 2004 ⁶⁶	
Hemagglutination (HA)				
	79%	81.1%	Bajani <i>et al.</i> 2003 ⁵⁹	
Lateral flow assay (LFA)				
1st week	52.9%	93.6%	Sehgal <i>et al.</i> 2003 ⁷¹	
2-4 weeks	86%	89.4%	Sehgal <i>et al.</i> 2003 ⁷¹	
Molecular methods	Real-time Polymerase Chain Reaction (RT-PCR)			
	rss gene	56	90	Thaipadunpanit <i>et al.</i> 2011 ⁵²
	lipl32 gene	43	93	Thaipadunpanit <i>et al.</i> 2011 ⁵²
		100	97	Thaipadunpanit <i>et al.</i> 2011 ⁵² , Villumsen <i>et al.</i> 2012 ⁵³

Rapid Antibody-Based Diagnostic Tests:

Microscopic Agglutination Test (MAT) is laborious

MAT, described by Martin and Pettit is the most widely used reference method for the diagnosis of human leptospirosis. The MAT requires maintaining a panel of live leptospires belonging to different serovars in the laboratory for the detection of agglutinating antibodies against *Leptospira* in the patient serum. The principle of MAT is simple, but this method is time consuming and laborious. Moreover, maintaining a live panel of *Leptospira* from different serovar in the laboratory may cause greater potential hazard to laboratory personnel. The sensitivity of the assay was 30% after 1 to 5 days which increases to 63% after 5 to 14 days of illness. In the convalescent stage, the sensitivity was shown to be 76-91.4%. The specificity of MAT assay was reported to be 86.7-97%.^{55,56} MAT cannot be standardised since live leptospires are used as antigens.⁴⁶

Enzyme-Linked Immunosorbent Assay (ELISA) is more specific

ELISA has been used extensively for the diagnosis of leptospirosis and it allows the detection of *Leptospira*-specific

IgM antibodies. Commercial IgM ELISA is based on the detection of antibodies against whole cell or recombinant surface protein or lipoprotein of *Leptospira*.⁵⁷ The cost of this diagnostic test is relatively cheap and requires only a small volume of sample for the assay. *Leptospira*-specific IgM may be detected four to five days after the onset symptoms before detecting *Leptospira*-specific IgG which persist for up to five months in patients.⁵⁸ The sensitivity and specificity of the IgM ELISA was reported to be 86.5% and 97% respectively.⁵⁹ Several studies indicate that ELISA is able to detect anti-*Leptospira* antibodies earlier than the MAT.^{13,60} The use of recombinant LipL32 in ELISA was able to detect *Leptospira*-specific antibodies with an excellent sensitivity (96.4%) and specificity (90.4%) and it is considered as a screening test for large number of serum samples.⁶¹⁻⁶⁴

Other serological tests: The immunofluorescence assay is based on the specificity of the antibody to their antigen and uses a fluorescent dye as detection reagent. This method can be used to detect specific antibodies in body fluids or antigen in tissue samples. The sensitivity of the immunofluorescence assay was reported to be 45.5 and 91.9% and the specificity

was 96.8 and 100%.^{65,66} Although the specificity and sensitivity of the immunofluorescence assay was comparable to ELISA, it is not widely available since it requires fluorescent microscope.⁶⁷ Haemagglutination assay uses erythrocytes, which are sensitized with an extract of an erythrocyte-sensitizing substance from *L. biflexa* serovar Patoc.^{68,69} The test involves incubating heat-inactivated test serum with sensitized erythrocytes which causes the formation of agglutination.⁴⁶ The concept of this assay is simple and requires no specialized equipment. The sensitivity and specificity of haemagglutination assay was reported to be 79% and 81.1% respectively.⁵⁹ It might be useful for laboratories which require high performance of leptospirosis diagnostic capability.⁷⁰ Lateral flow assay (LFA) requires no specialised equipment and electricity and detects the presence of target analytes in the sample. LFA can be performed at the bedside of the patients using whole blood and the results were comparable to ELISA and MAT.⁷¹⁻⁷³ LFA has sensitivity and specificity of 52.9% and 93.6% for the first week of illness and the levels increased to 86% and 89.4% after 2 to 4 weeks.⁷¹ LFA is not widely used since its usage is restricted to specific geographical locations.⁷⁴

Treatment and prevention of leptospirosis is available

It is important to treat leptospirosis early to avoid severe complication and potential fatality. Leptospirosis can be treated with antimicrobial therapy. There are several antibiotics including penicillin, erythromycin, ampicillin, amoxicillin, ceftriaxone and doxycycline. Patients with severe infections of leptospirosis should be treated by intravenous penicillin in doses of 50,000-100,000 U/kg/day for seven to ten days. Meanwhile, for patients with penicillin allergy, erythromycin may be given 30-50mg/kg/day in three to four doses for seven to 10 days. For mild cases, oral amoxicillin (30-40mg/kg/day) or ampicillin (50-100mg/kg/day) may be given to the patients four times a day for seven to ten days. Children with more than 8 years of age can be treated with doxycycline (2mg/kg/dose) two times a day for seven to 10 days.^{75,76} These antibiotics are able to decrease the fever and most symptoms. Leptospirosis patient should be observed for the evidence of renal failure and treated for dialysis when necessary. There is no vaccine currently available for human use. Preventive measures including vaccination program for domestic animals, establishing a rodent control program, practicing a good personal hygiene such as wearing footwear when outdoors and wearing gloves and face mask while working with animals are recommended.

CONCLUSION

Leptospirosis is a zoonotic disease which requires immediate treatment. Accurate diagnosis is challenging as the organism is not easily cultivable. There is a need for developing newer techniques for rapid detection of leptospirosis which require validation by reference centres that are familiar with validation schemes of diagnostic tests. The current diagnostic tests for Leptospirosis show varying levels of performance (Table II). The sensitivity and specificity of DFM is lower compared to all the other assays. Although the specificity of most of the serological tests is higher, the sensitivity is lower particularly during the first week of illness. Also, most serological tests including MAT assay is prone to subjectivity

as the results are read by visual observation. PCR has high specificity. However, it becomes negative after 2 weeks. The ELISAs show high specificity and sensitivity and they are not routinely used in many laboratories in Malaysia. Based on the current performance of the assays, combination of PCR and ELISA might yield rapid and accurate diagnosis and may be beneficial in outbreak investigations. Since these assays do not identify different serovars, positive samples may be tested by MAT for determining the serovar which is important for epidemiological investigations. Studies on mechanism of pathogenesis of *Leptospira* are also needed to understand the interaction of *Leptospira* with the host in causing the disease and protective immune responses to develop vaccines that are safe for human use. Preventive measures are required to control the spread of leptospirosis in the community.

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