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Leptospirosis: Increasing importance in developing countries

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ABSTRACT

Leptospirosis is a zoonotic disease caused by the pathogenic helical spirochetes, *Leptospira.* Symptoms include sudden-onset fever, severe headaches, muscle pain, nausea and chills. Leptospirosis is endemic in developing countries such as Malaysia, India, Sri Lanka, and Brazil where thousands of cases are reported annually. The disease risk factors include the high population of reservoirs, environmental factors, recreational factors, and occupational factors. To end the endemicity of leptospirosis, these factors need to be tackled. The management of leptospirosis needs to be refined. Early diagnosis remains a challenge due to a lack of clinical suspicion among physicians, its non-specific symptoms and a limited availability of rapid point-of-care diagnostic tests. The purpose of this review is to provide insight into the status of leptospirosis in developing countries focusing on the risk factors and to propose methods for the improved management of the disease.

1. Introduction

Leptospirosis is a zoonotic disease that was first identified in 1886 by Adolf Weil (Levett, 2001). Leptospirosis is caused by helical and highly motile spirochetes known as *Leptospira* (Picardeau, 2013). There are over 20 species of *Leptospira*, categorised into over 300 serovars grouped into more than 24 serogroups (Picardeau, 2013). A prominent species of the genus *Leptospira* is *Leptospira interrogans,* which comprises some of the pathogenic serotypes causing leptospirosis (Thayaparan et al., 2013). There are 12 other pathogenic species, six of which include *L. santarosai, L. weilli, L. alexanderi, L. kirschneri, L. borgpetersenii,* and *L. noguchii* (Ahmed et al., 2006). Meanwhile, *L. biflexa* comprises some of the saprophytic serotypes (Thayaparan et al., 2013). A more contemporary taxonomy classifies *Leptospira* into three phylogenetic lineages according to the degree of virulence of the species. These lineages include saprophytic, intermediate, and pathogenic (Perolat et al., 1998). Pathogenic species and intermediate species share a closely common ancestor, but the intermediate species exhibit moderate virulence in humans and animals (Perolat et al., 1998). A recent study on the genomics of *Leptospira* elucidated the discovery of novel leptospiral species, of which four were categorized as pathogenic, ten were listed as intermediates, and twelve were considered saprophytic. The pathogenic species were isolated from New Caledonia, Mayotte, Malaysia, and Ireland while the intermediate and saprophytic species were isolated from Japan, New Caledonia, Mayotte, and Malaysia (Vincent et al., 2019). The outer envelope of the *Leptospira* is composed of lipopolysaccharides and membrane proteins, and it surrounds the protoplasmic cell body (Haake and Matsunaga, 2011). The axial filament aids the motility of *Leptospira* while the hook is formed by presence of flagella at the cell terminus (Bromley and Charon, 1979; Raddi et al., 2012). Leptospirosis has a large global distribution due to a vast range of mammalian hosts, globalisation and climate change (Costa et al., 2015). An estimated 1.03 million cases occur annually, making it one of the most prominent zoonotic causes of morbidity and mortality (Costa et al., 2015). Despite high leptospirosis incidence in South and Southeast Asia, especially in countries with large populations like India and Indonesia, the disease remains under-reported due to poor surveillance (Costa et al., 2015).

Here we discuss the environmental, occupational, and recreational risk factors of leptospirosis in developing countries. In addition, the animal species that are the main reservoirs of this spirochete and the role they play in infecting humans will be explored. Furthermore, we will discuss the reasons why the disease is underreported besides explaining why early diagnosis is imperative. We examine the current

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level of public awareness and clinical suspicion and propose ways to ameliorate the standards of these aspects.

2. Methodology

A literature review was carried out on the overall epidemiology, historical background, risk factors, and diagnosis of leptospirosis. Peer-reviewed scientific articles obtained from PubMed and Lancaster University's database were consulted. The search terms utilized include “leptospirosis”, “Malaysia”, “India”, “Sri Lanka”, “Brazil”, “developing countries”, “risk factors”, “symptoms”, “serovars”, “reservoirs”, and “diagnosis”. The included references were those that focused primarily on leptospirosis in developing countries. Papers published in languages other than English were excluded. Additionally, the websites of public health organizations including the Centers for Disease Control (CDC) and World Health Organization (WHO) were referred to for its global burden.

3. Leptospirosis

Leptospirosis occurs when leptospires enter the body through the skin or mucous membranes of the mouth and conjunctiva. Transmission is either direct, from host to host, or indirect, via soil, infected animal urine or contaminated water (Haake and Levett, 2015). Humans may be accidental hosts and shed leptospires through urination (Haake and Levett, 2015). Once they enter the body, leptospires disseminate hematogenously and invade the host tissues (Wunder et al., 2016). Adherence is the first critical step for infection (Adler, 2014). Many leptospiral adhesins can adhere to a range of host components (Adler, 2014) and have shown the ability to evade the host immune system. For example, leptospires can evade the complement system by disabling complement proteins and enlisting host complement regulators (Fraga et al., 2016). The former is achieved when leptospires either inactivate the proteins using self-produced proteases or cleave them using acquired host proteases (Fraga et al., 2016). Leptospires recruit host complement regulators, which control complement initiation, by attaching to them through surface proteins. This approach allows the leptospires to interfere with the host's complement system pathways (Fraga et al., 2016). Leptospires may also directly evade macrophages and neutrophils until anti-leptospiral antibodies are produced (Banfi et al., 1982). Studies have proposed that leptospires can hide themselves within macrophages and later escape through apoptosis (Jin et al., 2009).

Leptospirosis is a notifiable disease in Malaysia. The number of cases is on the rise with 1976 cases estimated in 2010 with 69 deaths while 2268 cases and 55 deaths were estimated in 2011 (UKMMC, 2017). Tan et al. (2016) reported a sum of 3665 cases in 2012 which then increased to 4557 cases in 2013. Up to 72.3% of cases in those two years were diagnosed in males while 51.6% of reported cases involved those older than 30 years (Tan et al., 2016). The number of fatal cases declined to 48 in 2012 but rose to 71 cases in 2013 and 92 cases in 2014 (UKMMC, 2017). There were 7806 cases of leptospirosis in 2014 and 5370 cases in 2015 (Garba et al., 2017). There were 5284 cases and 52 deaths in 2016 (Bakar and Rahman, 2018). (Fig. 1) The staggering number of cases annually shows that leptospirosis is a formidable threat to public health and a better understanding of the disease must be cultivated.

Leptospirosis also places a significant burden on other developing countries in Asia and beyond. Thailand reports an incidence rate of several thousand cases annually (Cosson et al., 2014). The zoonotic disease is also prevalent in India, where there have been numerous outbreaks in the last decade (Bharadwaj et al., 2002; Jena et al., 2004; Supe et al., 2018). Leptospirosis has quickly become one of the most prominent threats to public health in Sri Lanka and is endemic in all but one district. A massive outbreak of the disease there in 2008 led to over 7000 reported cases (Warnasekara and Agampodi, 2017). Leptospirosis is also a prominent cause of febrile illnesses in South America. Every year, outbreaks occur in the urban slum areas in Brazil, primarily due to heavy rainfall, flooding, and poor sanitation (Jorge et al., 2017; Ko et al., 1999).

The symptoms of acute-phase leptospirosis include sudden-onset fever, severe headache, muscle aches, nausea, diarrhoea, vomiting and chills (Haake and Levett, 2015), however it is often misdiagnosed as dengue fever in tropical regions (Benacer et al., 2016). Despite mild initial symptoms, delayed intervention leads to severe and possibly fatal Weil's disease, characterized by haemorrhage, jaundice and renal failure (Bharti et al., 2003; Tan et al., 2016). Leptospirosis treatment mainly involves a range of antibiotics administered either orally or intravenously (Yaakob et al., 2015). Antibiotics include tetracyclines, penicillin and ceftriaxone (Brettmajor and Rodney, 2012). The most commonly used antibiotic is penicillin (Charan et al., 2013). Early administration of these antibiotics greatly reduces the risk of Weil's disease and mortality (Tubiana et al., 2013). More than 10^3 leptospires/mL of blood prior to the introduction of antibiotics is associated with a higher likelihood of severe leptospirosis (Tubiana et al., 2013). Once progressed to Weil's disease, it may require intensive care treatment including haemodialysis and blood transfusions. Severe pulmonary haemorrhages can only be managed with mechanical ventilation (Andrade et al., 2007; Ko et al., 2012; Goarant, 2016).

4. Risk factors of leptospirosis

4.1. Reservoirs

Reservoirs are any animals that remain relatively asymptomatic despite being infected. Reservoirs retain the leptospires within their renal tubules, where they thrive and multiply, and occasionally release some of them through urination (Goarant, 2016). The male and female genitals of reservoirs have also been implicated as important sites where leptospires can persist (Hamond et al., 2015; Silva et al., 2019) Humans can be infected directly through contact with the urine of infected reservoirs or indirectly through contact with the pathogen-contaminated environment (Goarant, 2016). Thayaparan et al. (2015) analyzed the link between wildlife reservoirs and leptospirosis seropositivity in humans by determining the common serovars in the communities near the forest reserves of Sarawak, where bats and rodents coexist. One-third of the participants were tested seropositive and those living nearby caves, where contact with wildlife is more common, showed higher seropositivity. This study demonstrated that exposure to reservoirs heightens the risk of infection. Furthermore, different organisms may be susceptible to different strains of the bacteria, due to some form of coadaptation. For instance, the serovar Canicola is linked with dogs while cattle are prone to sheltering and excreting the serovar Hardjo (Goarant, 2016).

In rural areas, where agriculture is prominent, mammals such as cattle, pigs, dogs and goats are potential vectors. Dogs can get infected when they encounter leptospires-contaminated water or infected urine, and pass the pathogens in their urine, putting their owners at risk (Gay et al., 2014). In Yucatan, dogs, cattle and pigs were recorded as the main reservoirs (Cárdenas-Marrufo et al., 2011). Tarassovi and Hardjo are the main serovars found in cattle while pigs carried the Bratislava and Icterohaemorragiae. Dogs predominantly carried the serovar Canicola. These findings may be relevant to other developing countries too and suggest that the domestic animals should be vaccinated in endemic countries to prevent the risk of transmission to humans.

In urban areas, rodents, particularly rats are the main reservoirs (Goarant, 2016). The main species of rat involved is the *Rattus norvegicus*, which harbors the serogroup Icterohaemorrhagiae (Goarant, 2016). Rodents can also be carriers for serogroups Copenhageni, Bim, and Ballum (Perez et al., 2011; Esfandiari et al., 2015). In Sri Lanka, rodents are the main reservoirs and their endemic serovar

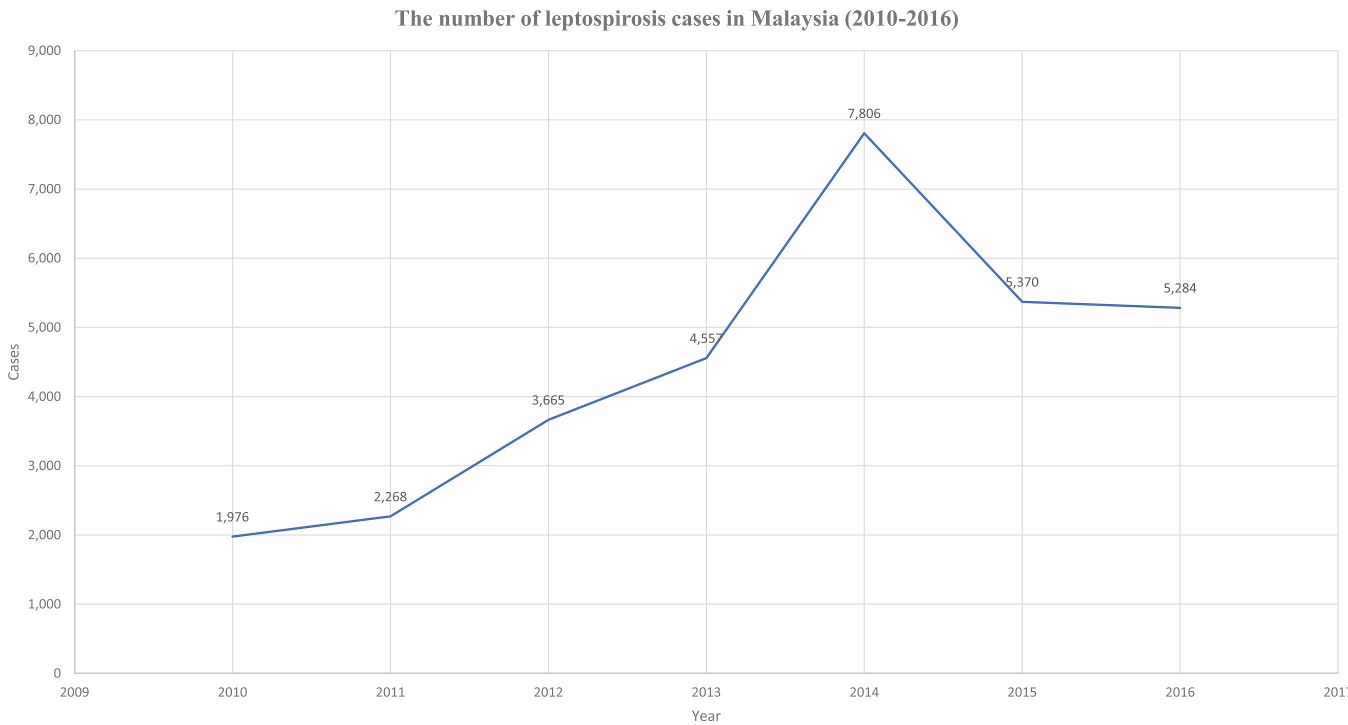

Fig. 1. A chart demonstrating the trend in the number of leptospirosis cases in Malaysia from 2010 to 2016 (Bakar and Rahman, 2018; Garba et al., 2017; Tan et al., 2016; UKMMC, 2017). The number of cases rose steadily from 2010 to 2013 before rising exponentially from 2013 to 2014. However, there was a notable decline in reported leptospirosis incidences from 2014 to 2015. From 2015 to 2016, the number of cases remained relatively constant.

is Icterohaemorrhagiae (Robertson et al., 2012). However, antibodies for other serovars like Serjoe, Icterohaemorrhagiae, and Cynopteri have also been found in the serum samples of infected Sri Lankan patients (Koizumi et al., 2009). In Malaysia, rodents, especially rats, are the main reservoirs of leptospirosis. There are approximately 248 million rats in Malaysia, approximating 8 rats per person (Lim, 2011). This problem is compounded by unsanitary and indiscriminate trash disposal from eateries, wet markets and homes (Garba et al., 2018). Although Benacer et al. (2013a) reported only 20 pathogenic cultures among 300 rats, the serovars detected were the highly pathogenic strains, Bataviae and Javanica. Hence, rodent population control is vital.

4.2. Environmental risk factors

Climate determines the dynamics, endemicity and spread of a disease as it affects the survival, growth and reproduction of pathogens (Gallana et al., 2013). Tropical countries struggle with various endemic infectious diseases, including leptospirosis, due the suitability of their climate (Gallana et al., 2013). The hot and humid weather in Malaysia accommodates for the continued survival of leptospires in the environment (Garba et al., 2018). Similarly, the tropical weather in Sri Lanka has been associated with the prevalence of leptospires and leptospirosis occurrence (Ehelepola et al., 2019). Shed pathogens can remain for weeks to months in warm and humid areas, away from direct sunlight (Goarant, 2016). Developed countries that are non-tropical may also be susceptible to leptospirosis after heavy rainfall. A post-race outbreak of leptospirosis in Martinique (Hochedez et al., 2011), the largest reported US outbreak in Illinois (Morgan et al., 2002), and an outbreak after a sports event in Florida in 2005 (Stern et al., 2010) occurred because there was heavy downpour prior to those events, which likely drained infected animal urine into bodies of water such as lakes, rivers or puddles (CDC, 2016).

Watson et al. (2007) investigated the relationship between natural disasters and disease epidemics. They concluded that the root cause of outbreaks is population displacement, whereby victims gather at evacuation centres for safety. Sanitation, public hygiene and healthcare availability become a challenge and the risk of an outbreak is greatly increased (Watson et al., 2007). Furthermore, the overflowing of drains and structural damage to sewers lead to water contamination, an avenue for water-borne pathogens, including *Leptospira* spp., to infect hosts (Assar, 1971). Floods heighten the possibility of a leptospirosis outbreak because the proximity of humans and rodents on higher ground facilitates the spread of the pathogen (Watson et al., 2007). Additionally, contaminated soil leaching, drain spillages, and the drifting of drowned vectors from flood sites further compound the risk of infection (Wasiński and Dutkiewicz, 2013; Garba et al., 2018).

In Malaysia, flooding is a recurrent phenomenon during the monsoon season. Some of the flood-vulnerable states include Pahang, Perak, Johor and Terengganu (Amin and Othman, 2018; Aziz et al., 2016; Sulaiman et al., 2010; Zahari and Hashim, 2018). After a major flooding event in 2015, Kelantan, Perak, Terengganu and Pahang reported a total of 647 suspected and 110 confirmed leptospirosis cases (Yaakob et al., 2015). The association between floods and leptospirosis outbreaks applies to India as well. After a cyclone hit the state of Orissa in 1999, an outbreak was reported (Sehgal et al., 2002).

Public hygiene also plays a role in the spread of diseases. Around 1.9 million deaths worldwide and 4.2% of the global disease burden have been linked to poor sanitation (World Health Organization, 2009). Leptospirosis is prevalent in poorer urban areas that face the effects of poor sanitation. Set to increase to a whopping 1.4 billion people by 2020, the urban slum population faces constant exposure to the pathogens resulting from inefficient management and surveillance (Abela-Ridder et al., 2010; UN Habitat, 2012). In Malaysia, urban poverty occurs due to the unequal distribution of wealth and development. Economical divides exist within the same area in Kuala Lumpur (Siwar and Kasim, 1997). Large populations make it difficult to manage sanitation, leading to poor garbage management and an increase in leptospirosis vectors (Maciel et al., 2008).

4.3. Occupational risk factors

Occupational risk factors involve either direct or indirect exposure. Direct exposure occurs less often and usually involves occupations that require interaction with potentially-infected animals or the pathogen itself (Haake and Levett, 2015). Leptospirosis can be transmitted through animal milking and exposure to infected cadavers (Wasiński and Dutkiewicz, 2013). Those who risk indirect exposure include farmers, fishermen, garbage collectors, and sewage cleaners (Goarant, 2016; Brightman, 2018). Scratches or cuts from hard labor provide entry routes for leptospires into the body when workers are not provided the necessary safety equipment like gloves and rubber boots (Kamath et al., 2014).

Over 50% of reported leptospirosis cases in Bulgaria in 2005 and 30% of cases in France and Germany were occupationally-linked (Jansen et al., 2005; Taseva et al., 2007; Abgueguen et al., 2008). Paddy farmers are the most at-risk occupational groups in Sri Lanka, constituting over 43.5% of patients in 2007. Meanwhile, Malaysian agricultural workers are high-risk individuals for leptospirosis. Rafizah et al. (2013) found that agriculture-associated cases were the highest in number. The oil-palm plantation workers recorded the highest seroprevalence (Garba et al., 2018). Ridzuan et al. (2016) corroborated this finding, calculating a seroprevalence of 28.57% among 350 oil palm plantation employees. Cattle rearing also places workers at risk, especially when garbage dumps are close by to some farms, as reported by 49.2% of the cattle farmers in a survey (Daud et al., 2018). The potential vectors that come with this problem heighten the probability of finding leptospires in the environment by nearly 2.5 times (Daud et al., 2018). Jobs that involve waste and rubbish management also pose risks. Samsudin et al. (2015) found that Selangor trash collectors had a seroprevalence of 41.5%, followed by cleaners at 33.3%. Trash collectors and cleaners in north-eastern Malaysia recorded seroprevalences of 27.4% and 26.0%, respectively (Shafei et al., 2012).

4.4. Recreational risk factors

In the United States, leptospirosis was considered mostly an occupational hazard before the 1970s until there was a shift towards recreational transmission in the late 1970s (Martone and Kaufmann, 1979). Over the years, many countries worldwide also experienced the transition due to globalisation and an increase in the popularity of outdoor activities (Tilahun et al., 2013). Recreational risk factors are closely associated with the environment as the latter harbors the causative agents. *Leptospira* spp. can survive for long periods of time in the hosts' renal tubules and enter the environment, where they thrive, when the hosts urinate (Monahan et al., 2009). Participants of outdoor activities will encounter these environmental elements (Monahan et al., 2009). During these events, injuries, including small cuts, provide entry for the leptospires into the body, where they cause illness. Spirochetes can even infect someone without cuts by breaching mucosal surfaces or skin surfaces after prolonged exposure (Waitkins, 1986; Faine, 1999).

Sports and recreational activities that have led to participants contracting the disease include triathlons, white water rafting, canoeing, caving, swimming, and racing. One such recreationally-linked incident is the Springfield triathlon, whereby 66 participants were diagnosed with leptospirosis after many accidentally swallowed lake water while swimming (Morgan et al., 2002). Similarly, a 36-year-old Costa Rican woman contracted the illness after surviving a near-drowning incident while white water rafting (Gelman et al., 2002). In Sri Lanka, 20 office employees fell ill with the disease after a staff outing that involved white-water rafting, the first recreationally-linked leptospirosis outbreak in that country while a canoeing competition in Ireland also resulted in numerous cases (Boland et al., 2004; Agampodi et al., 2014). The diversity of these locations and their climates tell us that leptospirosis is not exclusively a tropical disease, especially when recreational risk factors are involved. Nevertheless, the disease burden still rests more heavily on the shoulders of developing and tropical countries.

In Malaysia, an American caver contracted the illness in 1994 after a caving expedition in Gunung Buda, Sarawak (Mortimer, 2005). It is possible that the cave water had been contaminated by the infected urine of bats and rats commonly found in the area (Smith et al., 1961). The Sabah Eco-Challenge multisport event in 2000, which involved outdoor activites including jungle-trekking, swimming and cave exploration, left 42% of attendees with leptospirosis likely due to injuries and prolonged exposure to contaminated water or soil (Sejvar et al., 2003).

5. Diagnosis of leptospirosis

5.1. Clinical diagnosis

There is a high chance of clinical misdiagnosis, especially in tropical regions with other endemic diseases (Rafizah et al., 2012). Lack of clinical suspicion towards leptospirosis is one reason why zoonotic diseases have been re-emerging in industrialized countries (Cutler et al., 2010). In Malaysia, a febrile case might more likely be suspected as dengue fever than leptospirosis as both acute-phase illnesses display non-specific symptoms (Bharti et al., 2003; Garba et al., 2017). A landmark symptom of leptospirosis is conjunctival suffusion, and this should play a bigger role in its diagnosis to avoid misdiagnosis (Zaki and Shanbag, 2010). Furthermore, rashes are more unique to dengue fever (Zaki and Shanbag, 2010). However, this should not be a definitive marker because anomalies are possible (Musso and La Scola, 2013). In other developing countries like Brazil, the disease could be confused with Zika, chikungunya, and yellow fever due to their similar clinical presentation (Patterson et al., 2016). Thus, conclusive testing involving laboratory diagnostics is vital.

5.2. Laboratory diagnosis

5.2.1. Culture

Culture is a routinely-used diagnostic in countries like Malaysia (Ministry of Health Malaysia, 2011). It involves the isolation and growth of leptospires (Wuthiekanun et al., 2007). Blood and cerebrospinal fluid (CSF) samples are cultured within the first 10 days while urine samples can be cultured after the second week (Musso and La Scola, 2013). The urgency for blood culturing once symptoms appear is because leptospiraemia, which means having *Leptospira* in the blood, occurs before that and only lasts for a while (Haake and Levett, 2015). It is straightforward and media inoculation can be carried out by the patient's bedside (Turner, 1970; Musso and La Scola, 2013). Urine cultures pose a challenge because they are susceptible to contamination and excreted urine is toxic to the leptospires' survivability (Musso and La Scola, 2013). Hence, urine culture media are often supplemented with antimicrobials (Haake and Levett, 2015).

While culture could be ameliorated using spun plasma or whole blood, resources for it are not widely available (Wuthiekanun et al., 2007; Lim, 2011). In Malaysia, it is only available at the Institute of Medical Research (IMR) in Kuala Lumpur (Ministry of Health Malaysia, 2011). Additionally, leptospires require special media like Ellinghausen, McCullough, Johnson, and Harris medium (EMJH) and Fletcher's media to grow (Lim, 2011). The slow growth of *Leptospira* delays diagnosis considerably (Lim, 2011). The leptospiral-inoculated semisolid media need to be incubated for six to 13 weeks in the dark, between 28 °C and 30 °C, and examined periodically using darkfield microscopy (Farr, 1994; Musso and La Scola, 2013). Furthermore, pathogen isolation must be done before antibiotic treatment (Musso and La Scola, 2013). Hence, the limitations of culture outweigh its benefits (Table 1).

Table 1
A summary of the advantages and disadvantages of the laboratory diagnostic tools used for the diagnosis of leptospirosis in Malaysia.

Diagnostic	Advantages	Disadvantages	References
Culture	Straightforward and can be carried out at patient's bedside Can identify the specific strain of infecting leptospires, useful for epidemiology	Urine cultures susceptible to contamination Not widely available locally Special media is required Isolation must be done before antibiotic treatment Leptospira grows slowly Inoculated media must be incubated at length in the dark, between 28 °C and 30 °C	Turner (1970); Musso and La Scola (2013) Ministry of Health Malaysia (2011); Haake and Levett (2015) Lim (2011) Musso and La Scola (2013) Lim (2011) Farr (1994); Musso and La Scola (2013)
Polymerase chainreaction (PCR)	More sensitive than culture qPCR combines amplification and quantification Positive results are likely during the first week; allows for early diagnosis Can detect DNA of antibiotic-killed organisms	Cannot identify the specific pathogen strain May not be as accurate once anti-leptospiral antibodies are produced	Haake and Levett (2015) Boonsilp et al. (2011)
Enzyme-linkedimmunosorbentassay (ELISA)	Genus-specific IgM-ELISA is appropriate for acute-phase diagnosis ELISA can be widely found Does not require much expertise Results obtained after a few hours	Genus-specific IgM-ELISA cannot determine pathogen serogroup IgM-ELISA is most accurate only during the convalescent phase IgM-ELISA may not be as accurate in leptospirosis-endemic countries Conclusive diagnosis might require MAT	Musso and La Scola (2013); Niloofa et al. (2015) Budihal and Perwez (2014); Desakorn et al. (2012); Ministry of Health Malaysia (2011) Desakorn et al. (2012); Picardeau et al. (2014) Picardeau et al. (2014)
Microscopic agglutination test(MAT)	Suitable for epidemiological purposes High specificity Can be paired with ELISA for comprehensive testing Can cross-validate ELISA findings	Cautious interpretation is necessary in leptospirosis-endemic countries as positive results may reflect former infections Sensitivity not very high and is lower than that of ELISA Cannot determine *Leptospira* serovars Requires the maintenance of live *Leptospira* cultures; poses health risks for workers Time-consuming and elaborate	Cumberland et al. (2001); HYPERLINK \l "bib4" Agampodi et al. (2016); Murray et al. (2011) Murray et al. (2011), Yaakob et al. (2015). Eugene et al. (2015) Yaakob et al. (2015); Rafizah et al. (2013) Ministry of Health Malaysia (2011)

5.2.2. *Polymerase chain reaction (PCR)*

PCR, which yields rapid results, is a preferable alternative to culture (Valones et al., 2009). Early diagnosis is crucial for leptospirosis, so that antibiotics can be administered effectively (Musso and La Scola, 2013). Samples for leptospirosis PCR can be obtained from the blood, CSF, and urine (Yaakob et al., 2015). Both PCR types detect the leptospires using primers that target either bacteria-specific genes, such as *rrs* and *secY*, or pathogenic Leptospira-specific genes, such as *lipL32, ligA*, and *ligB* (Mérien et al., 1992; Levett et al., 2005; Ahmed et al., 2009). Real-time PCR (qPCR) can also be conducted using primers designed against the conserved regions of the *lipL32* sequences of all pathogenic *Leptospira* species (Levett et al., 2005).

A disadvantage of PCR is that it can only quantify leptospires, but it cannot identify the specific strain, which is important in epidemiological surveillance, unlike culture (Haake and Levett, 2015). However, Esteves et al. (2018) found that performing high-resolution melting analysis (HRM), a genotyping method used to analyse DNA fragment by determining the correlation between temperature and denaturation to study the nucleic acid sequences, after qPCR enabled the serovars of leptospires to be identified (Tong and Giffard, 2012; Naze et al., 2015). Additionally, post-PCR sequencing or DNA-based barcoding could be conducted to acquire molecular data that aids species identification (Hebert et al., 2003). This can be achieved with a short designated DNA sequence of a gene target being utilized as a discriminatory taxonomic barcode (Hebert et al., 2003).

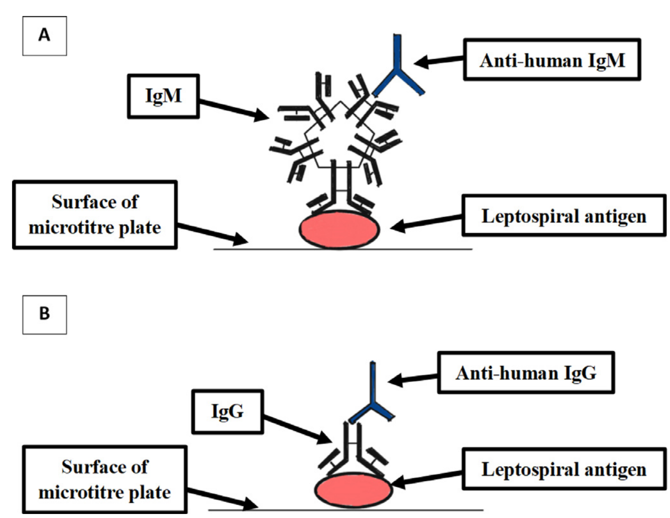

Fig. 2. A simplified illustration of the direct ELISA in the context of leptospirosis diagnosis. (A) Direct ELISA for the detection of anti-leptospiral IgM. The IgM binds to the leptospiral antigen in an antibody-antigen interaction while the anti-human IgM binds to the antigen-bound IgM to elucidate detection (Honarmand and Eshraghi, 2011). (B) Direct ELISA for the detection of anti-leptospiral IgG, which binds to the leptospiral antigen, and is in turn bound by anti-human IgG for its detection (Honarmand and Eshraghi, 2011).

5.2.3. *Enzyme-linked immunosorbent assay (ELISA)*

For leptospirosis diagnosis, ELISA can be used to detect immunoglobulin M (IgM) or immunoglobulin G (IgG) (Natarajaseenivasan et al., 2004) (Fig. 2). Nonetheless, it is the detection of IgM that is most vital for the diagnostic purposes of acute and subacute infections (Doungchawee et al., 2008). Numerous commercial IgM-ELISAs have been developed. Although most of them use the non-pathogenic *Leptospira biflexa*, this species carries cross-reactive surface antigens, such as lipopolysaccharides, thus enabling the detection of pathogenic species (Picardeau et al., 2014; Khaki, 2016). As IgM antibodies appear at low levels during the first week of illness, diagnosis confirmation using IgM-ELISA is most accurate after the second week, during the convalescent phase (Budihal and Perwez, 2014). However, it is still highly reliable to be conducted during the acute phase for early diagnosis (Niloofa et al., 2015). ELISA that detects genus-specific IgM will be more appropriate for acute-phase diagnosis as these antibodies appear earlier than their serovar-specific counterparts, but it cannot be used to determine the serogroup of the pathogen (Musso and La Scola, 2013; Niloofa et al., 2015).

IgM-ELISA might not be as accurate in leptospirosis-endemic countries because a subset of the healthy population may be seropositive (Desakorn et al., 2012). However, ELISA is a very promising tool as it can be found in laboratories across many tropical countries (Desakorn et al., 2012).

5.3. Microscopic agglutination test (MAT)

The gold standard of serological testing, the MAT detects leptospirosis by detecting the ability of IgM and IgG in patients' serum to agglutinate leptospires (Ahmad et al., 2005; Goarant, 2016). These antibodies become detectable between the sixth and tenth day and peak up to a month later (Ahmad et al., 2005). A variety of *Leptospira* reference strains can be used as antigens if the endemic serogroups within an area are unknown (Goarant, 2016). Serially-diluted serum samples are added to these cultures before microscopic observation for agglutination (Ehsanollah and pour Gholam, 2011). A positive leptospirosis diagnosis requires a four-fold rise in the agglutination end-point titre, which is the highest serum dilution that leads to at least 50% of the leptospires being agglutinated (Ehsanollah and pour Gholam, 2011; Perdhana et al., 2018). For more conclusive results the MAT should be conducted with paired sera (Haake and Levett, 2015). Cumberland et al. (2001) states that the antibodies remain in the body long after infection, so a MAT-positive result could reflect a previous infection. Hence, the diagnostic titre in leptospirosis-endemic countries must be significantly higher, up to 1/800 (Agampodi et al., 2016). Eugene et al. (2015) reported the sensitivity of MAT as 91.4% and its specificity, defined as the capability of a test to pinpoint the absence of a disease, as 86.7%, but because it detects late-appearing antibodies, it is more suitable for epidemiological purposes than for acute diagnosis (Lalkhen and McCluskey, 2008; Murray et al., 2011). MAT cannot be used to determine the infecting serovars (Murray et al., 2011).

The main challenge of the MAT is the maintenance of live *Leptospira* cultures, which are difficult to grow in a laboratory (Yaakob et al., 2015). It is time-consuming and elaborate besides posing health risks for those working with its live and virulent pathogens (Yaakob et al., 2015). Nonetheless, it can be paired with ELISA for a more comprehensive testing (Rafizah et al., 2013). MAT results tend to be negative during the disease's early phase, but it is still vital for cross-validating ELISA findings and averting false positives (Ministry of Health Malaysia, 2011; Picardeau et al., 2014).

6. Discussion

To successfully tackle the endemicity of leptospirosis, its prevention and management must be married. Efforts must be taken to prevent its spread altogether. The public must be aware of the disease's dangers and the ways to prevent it. The onus for closing the knowledge gap is on numerous groups including government bodies, medical officials, and the informed public. With the current accessibility to information, educating others and correcting their misconceptions have never been easier. Educating employees is vital as over 67% of town service workers had minimal knowledge about leptospirosis while 16.8% had never heard of it (Azfar et al., 2018).

Medical personnel, veterinarians, wildlife specialists and public health personnel should be involved in planning an integrated approach to tackling this issue. Engagements with state government officials is also necessary to educate them on the risks and damaging impact of leptospirosis so that state-wide prevention initiatives can be funded. State laws, such as the prohibition of indiscriminate rubbish dumping, must be more strictly enforced. State recreational parks, often implicated in outbreaks, should be kept clean to avoid potential breeding grounds for leptospirosis reservoirs (Ridzlan et al., 2010; Benacer et al., 2013b). The Eco-Challenge outbreak is a sober reminder that both sporting event organizers and local authorities need to collaborate to ensure the safety of competitions. Public health officers can encourage local representatives to collaborate with community leaders in organizing grass-roots awareness campaigns, during which residents can be enlightened about the disease (Liyanatul et al., 2016).

Municipal councils ought to support solid waste management in towns (Manaf et al., 2009). Hopefully through the raising of awareness on the state and federal level, if not through the enforcement of new occupational safety laws, employers will begin to shoulder the responsibility of ensuring that workers at risk of *Leptospira* exposure are always provided with the necessary equipment like rubber boots and gloves (Kamath et al., 2014).

The end goal of leptospirosis management is its successful treatment, which is quite straightforward if done early (Tubiana et al., 2013). This hinges on early diagnosis, so steps to enhance the likelihood of acute-phase diagnosis will greatly improve the disease outcome (Toyokawa et al., 2011). Both clinical diagnosis and diagnostic research have much room for improvement in developing countries. The former is challenged by the lack of clinical suspicion of leptospirosis among physicians (Cutler et al., 2010). In Malaysia, the tendency to associate leptospirosis symptoms with the more well-known dengue fever delays correct treatment and risks a more severe outcome (Guerra, 2013). Therefore, physicians should be updated on current leptospirosis trends and urged to increase their suspicion of it.

Laboratory tests are still required for disease management. As they are often laborious or time-consuming, there is a gap for readily available and highly-accurate point-of-care (POC) rapid tests, whereby patients can be tested and receive their diagnosis in one sitting. In ruling out dengue fever, physicians have the benefit of using commercial POC tests that yield results in minutes, but not when it comes to diagnosing leptospirosis (Wang and Sekaran, 2010; Krishnananthasivam et al., 2015). Having a convenient form of testing encourages better disease management through clinical suspicion, especially in rural areas where laboratory resources may not be readily available (Krishnananthasivam et al., 2015).

There have been a few rapid tests developed including the LeptoTek Lateral Flow, the Leptocheck-WB, Leptorapide®, and VISITECT®-LEPTO (Goris et al., 2013; Chang et al., 2014). The latter two show to have below-average diagnostic values, leading to misdiagnosis and inconclusive results (Chang et al., 2014). The first three rapid tests show better sensitivity with paired sera, but still require confirmation with laboratory tests (Goris et al., 2013). Therefore, further research to develop accurate clinical POC rapid tests for leptospirosis is imperative (Lizer et al., 2017). As current tests involve detecting anti-leptospiral antibodies, there is a need to develop POC tests that detect the infecting leptospires' DNA or antigens in the blood, the same targets of molecular laboratory tests (Boonsilp et al., 2011). Such tests could be more accurate for diagnosing acute leptospirosis (Budihal and Perwez, 2014). Nevertheless, it is imperative to note that the fundamental treatment of leptospirosis should comply with the CDC recommendation that patients be treated on the sole basis of clinical suspicion, without awaiting laboratory results (CDC, 2018).

7. Conclusion

A potentially fatal disease, leptospirosis has been garnering more attention due to the growing number of cases worldwide. In developing countries, many cases are reported along with fatalities annually, indicating that the disease's endemicity has not relented. While one could blame the various risk factors that predispose the public to this disease, the very core of this issue stems from a poor understanding of the disease in developing countries. Without being educated on the danger of leptospirosis, its symptoms, routes of infection, and risk factors, the public will not realize the urgency to act in preventing its occurrence. Only when awareness is achieved can proactive steps be initiated and come to fruition. The population of reservoirs, particularly rats, is the primary risk factor that needs to be tackled by both citizens and the governing bodies. As for the diagnosis of the disease, physicians must be encouraged to sharpen their clinical suspicion of leptospirosis so that effective treatment can take place swiftly. Additionally, efforts need to be made to improve the current availability and accuracy of diagnostic tools. Therefore, research and development in this area should be encouraged through better resources and funding.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2019.105183.

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