

REVIEW ARTICLE

Leptospirosis, an emerging zoonotic disease in Malaysia

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Abstract

Leptospirosis is an endemic disease in Malaysia and recently has received increasing attention mainly due to several recent incidents that have resulted in human mortality which have alarmed health professionals in Malaysia. The increasing incidence of leptospirosis in forested regions is associated with the bacteria infecting small wild mammals other than rats. Infection in wildlife could result in the introduction of new serovars to humans and domesticated animals. More research on leptospirosis and the screening of wildlife and humans near wildlife habitats is required to have a better understanding of the involvement of wildlife in the disease.

Key words: infectious disease, leptospirosis, zoonotic disease, wildlife

INTRODUCTION

Leptospirosis, caused by infection with *Leptospira interrogans*, is a worldwide disease affecting both humans and animals resulting in morbidity and mortality.¹ The agent can be transmitted both directly and indirectly.^{2,3} In 1917, the role of rats as a source of human infection was first discovered,^{4,5} and subsequently some previous studies have demonstrated that other wild mammals can also act as potential carriers,^{6,7} including flying foxes.^{7,8} However, to date there has been little research conducted on the role of wildlife in outbreaks. As a result of the current significant level of deforestation and the increasing anthropogenic activities in the forested habitats and jungle, humans are at a greater risk of being exposed to new serovars of leptospires. Leptospirosis in wildlife can have negative consequences for biodiversity, human and livestock health, animal welfare and the economy of a country.^{9,10} This review will examine the impact of leptospirosis on wildlife, the current status of surveillance and the options to strengthen policies in Malaysia.

Morphology and taxonomic classification

Leptospires are spirochaetes in the order Spirochaetales, family of Leptospiraceae and

include two genera, *Leptospira* and *Leptonema*.¹¹ *Leptospira* are obligate aerobes with an optimum growth temperature ranging from 28°C to 30°C.^{12,13} The genus *Leptospira* was divided into two species based on serological classification: *Leptospira interrogans*, which comprises all the pathogenic strains and *Leptospira biflexa*, the environmental saprophytic strains.¹⁴⁻¹⁶ Based on the microscopic agglutination test (MAT), leptospires can be further divided into over 250 serovars. Serovars that are antigenically similar have been aggregated into serogroups.¹⁴ Furthermore both pathogenic and non-pathogenic serovars can be classified within the same species. A combination of methods is now used to confirm the species of *Leptospira*.

Pathogenesis

The most adverse clinical and pathological signs usually occur in young animals, especially when their maternally-derived immunity is waning.¹⁷ However, the common signs of *Leptospira* infection in farm animals are abortions, stillbirths, decreased milk production and a failure to thrive.¹⁸⁻²⁰ In all species, congenital infection and its sequelae are well reported. The most important difference between infection of animals and humans is the presence of chronic carriers

in animals through reservoirs in the kidneys and genital tract.²⁰⁻²³

Epidemiology of leptospirosis

Leptospire are widespread and their abundance is due to their ability to infect a range of animal species, including humans, as well as the ability to survive outside the host, if environmental conditions are favourable.²⁴⁻²⁶ The main sources of infection are urine of infected or carrier animals, contaminated surface water, mud and soil.⁵ Transmission can happen as a result of direct or indirect contact with infected animals or their secretions.⁵ The major route of infection is via mucous membranes, however carnivores can be infected through the ingestion of leptospiral-infected carcasses.²⁷ Usually leptospire appear in the blood four to 10 hours after infection and can be detectable in the blood from a few hours to seven days.²⁸⁻³⁰ Clinical signs may not occur in every case but severe fever is an important sign of acute leptospirosis. Animals that have recovered from leptospirosis may become carriers with the organism present in the renal tubules for periods of days to years,^{31,32} and subsequently leptospire are shed in urine into the environment.³²

Impact on humans, domestic animals, wildlife and ecotourism

Infectious diseases are transmitted globally through animal and human movements due to eco-tourism, wildlife research, reintroduction, rehabilitation, hunting, pet trade, laboratory and food industry demands and farming.^{33,34} These movements and activities are major contributing factors for the transfer of leptospirosis to animals and humans and the spread of the disease to new areas.

Research on leptospirosis has highlighted that rodents (20%), marsupials (35%) and bats (35%) are most likely to spread the disease.³⁵ Marsupials and chiropterans have been implicated as more significant reservoir hosts of leptospire pathogenic to humans than previously was recognized.³⁵ Studies on leptospirosis involving bats have produced equivocal results,⁷ however the fact that leptospire can potentially be spread through bats is a major concern due to their abundance, their ability to travel long distances and their potential to expose humans to infected urine. Ground-dwelling species, such as rodents foraging under bat roosts, could also encounter *Leptospira*-contaminated urine and spread the organism into urban areas.³⁶ From the limited

research on wildlife, several pathogenic serovars have been isolated with the most prominent serovars including *grippityphosa* and *pomona* from white-tailed deer (*Odocoileus virginianus*) in North America.³⁷ Surveillance of leptospirosis is important to determine the emergence of new strains, which have the potential to cause an outbreak. Although it is not economically viable to survey all wildlife species, it is important to regularly screen particular species, which live on the periphery of forests and have the potential to interact with humans (e.g. wild rats, carnivores and bats). Screening these 'sentinels' provides useful cost-effective information on the health status of a broader range of species.

Leptospirosis can cause serious economic loss to the livestock industries,^{38,39} and is a major cause of abortions in cattle.^{40,41} As the disease is common in domestic animals and wildlife, control in domestic animals can also have a positive impact on the disease in wildlife.

Worldwide there have been more than 184 distinct serovars of *L. interrogans* belonging to 20 serogroups identified.⁴² In Southeast Asia, the most common serovars associated with disease of domestic animals and humans are *icterohaemorrhagiae*, *autumnalis*, *canicola*, *pomona*, *patoc*, *grippityphosa*, *australis* and *poi*.^{43,44} The first three are the most important serovars with respect to veterinary and public health perspectives. Fever is an important and common presentation of tropical diseases and may sometimes be the only manifestation of a serious illness. Diagnosis is often difficult because of the many diagnostic possibilities, and symptoms, which may often be non-specific, and because of many doctors' unfamiliarity with the spectrum of tropical diseases. Treating the disease appropriately will reduce the risk to public health, and this can only come following a correct diagnosis. With the recent popularity of adventure travel and triathlete competitions, there is an increased awareness of the potential for leptospiral infection amongst those who partake in such activities.^{45,46}

Laboratory diagnostic methods used in Malaysia to diagnose leptospirosis

Diagnosis of leptospirosis depends on adoption of appropriate laboratory methods as the clinical presentation can vary greatly. The diagnostic method selected depends on the available samples and the purpose of testing. Identification of the infecting serovar is important

both epidemiologically and clinically, since this may assist in determining the source and likely outcome of infection. Different assays have been developed in an attempt to diagnose leptospirosis accurately, but the majority are unsuitable for use in developing countries due to their requirement for the maintenance of multiple strains or expensive equipment. Several methods are used in the diagnosis of leptospirosis in Malaysia. These include the simple Microscopic Agglutination Test (MAT); Polymerase Chain Reaction (PCR) test, as well as the Enzyme-linked Immunosorbent Assay (ELISA). The MAT is inexpensive, but time consuming and laborious, and is only carried out at the Institute for Medical Research (IMR) Kuala Lumpur, MKAK (Makmal Kesihatan Awam Kebangsaan) Sungai Buloh Malaysia for community outbreaks and also at some universities undertaking research on leptospirosis.⁴⁷ ELISAs are performed at hospitals with the necessary facilities and the IMR and MKAK. Currently culture of *Leptospira* is only performed at the IMR.⁴⁷ Diagnosis of animal leptospirosis is only undertaken at the Veterinary Research Institute, Ipoh and the Wildlife Department, Kuala Lumpur (PERHILITAN). Environmental samples can be tested in MKAK, Sungai Buloh, Malaysia.⁴⁷

Treatment, control and prevention of leptospirosis

A range of antibiotics are used to treat hosts with leptospirosis, with IV C-Penicillin (2M units 6 hourly for 5-7 days) being commonly used in severe adult human cases.^{47,48} The less severe cases are usually treated orally with antibiotics such as doxycycline, tetracycline, ampicillin or amoxicillin.⁴⁷ A trial on the penicillin derivative, doxycycline, on the prevention of infection and clinical disease was conducted in the North Andaman Islands in 1999.⁴⁹ Although the findings indicated that doxycycline prophylaxis did not prevent leptospiral infection in an endemic area, such treatment did have a significant protective effect in reducing the morbidity and mortality during outbreaks.

Cases of naturally occurring leptospirosis in wildlife have not been documented and little is known about the immunological features of the disease in wild mammals. However researchers have demonstrated antibodies to several serovars that have been found in a range of wildlife species (non-human primates, bats, rats, squirrels and mongoose) in Kuching Sarawak,

Malaysia.³ These animals are capable of being infected with one or more leptospiral serovars and can then serve as reservoir hosts for these serovars. These animals have an asymptomatic and transient infection with no obvious clinical signs of disease.

History of leptospirosis and geographical distribution

Leptospirosis has been documented worldwide, but formal reporting systems vary widely.^{2,50,51} Frequently the disease gains public attention when outbreaks occur in association with natural disasters, such as flooding in Nicaragua in 1995 or among foreign travellers and extreme athletes.⁵² Southeast Asia is an endemic area for leptospirosis, and infection in humans has been reported throughout the region. Seventy percent of the major pathogenic serovars have been isolated from Asia.⁵³ Although leptospirosis has been around for millions of years,⁵⁴ it is only in recent times that the bacterial cause of this disease has been identified. In 1886 Adolf Weil published an account detailing the icteric form of leptospirosis, which has since taken on his name.⁵⁴ However, his observations had already been postulated by others, including Hippocrates.⁵⁴ It has been hypothesised that leptospirosis was responsible for an epidemic among the natives of the Massachusetts coast just before the arrival of the Pilgrims in 1620.⁵⁵ What Weil described was the icteric form of leptospirosis with jaundice. In contrast the milder forms were hard to diagnose due to a lack of advanced bacteriology. Spirochaetes continued to cause problems for the later part of the 19th century, and it was only in 1907 that Stimson managed to isolate a leptospire from a patient.⁵⁶ His subsequent research highlighted that the bacteria were concentrated in the renal tubules and were shaped like question marks. This gave rise to the name "Spirocheta interrogans", which has remained.⁵⁶

Status of leptospirosis in South East Asia

Leptospirosis is emerging as a serious concern to public health in Southeast Asia.⁵⁷⁻⁵⁹ The disease has been recognised in patients from Indonesia with clinical jaundice and non-malarial fever, patients with clinical jaundice from Laos and Vietnam, and patients with haemorrhagic fever in Cambodia.^{53,60} In addition, a cross-sectional community-based study was conducted in Laos to obtain estimates of the background

sero-prevalence. These findings suggested that leptospirosis was under-reported in Southeast Asia due to the wide range of clinical symptoms associated with acute leptospiral infection.⁶⁰

Easton,⁶¹ reported a spike in infection in Manila following flash floods during the monsoon season. The bacteria would most likely have entered the patients' bodies through cuts on the skin when they waded barefoot in the floodwaters. Residents in low-lying areas and rice farmers were identified as a high-risk group for infection. One possible source of the problem was the increasing rat population with farmers from one province culling approximately 800 rats per night in their rice fields during the dry season.⁶¹ Studies in various locations in Thailand found that, after rats, dogs were the most important reservoir for leptospirosis with a seroprevalence of 11%, with the Bataviae serogroup being the most prevalent (5.2%).⁶² Playing in sewage, staying outdoors more than half the time and consuming raw meat increased the likelihood of sero-positivity in the sampled dogs. It was possible that the dogs acquired their infection from rats, which were responsible for an outbreak in north-eastern Thailand between 1999 and 2003.^{62,63} During that period, Thaipadungpanit *et al*,⁶³ developed a scheme of multi-locus sequence typing of pathogenic *Leptospira* and confirmed serovar Autumnalis was responsible for the outbreak and was being maintained by *Bandicota bengalensis* (bandicoot rats).⁶³

Besides infecting local residents, *Leptospira* can also cause illness in visitors to tropical regions, particularly those associated with eco-tourism and adventure travel.⁶⁴ Van Crevel *et al*,⁶⁴ investigated leptospirosis in 32 Dutch travellers between 1987 and 1991 and found 28 of them had returned from Southeast Asia with the majority visiting Thailand and 21 of these had taken a rafting tour. From this research it was recommended that doctors should consider leptospirosis in their differential diagnosis whenever a patient with fever returned from the tropics. Due to the increasing number of reported cases of leptospirosis in the western world, it has been recommended that travellers try to avoid high-risk aquatic activity in the tropics and undertake chemoprophylaxis if they do take part in water-sports in Southeast Asia.^{5,65} Saunders⁶⁶ examined 78 cases of leptospirosis in Pahang and reported the lack of specificity of the clinical syndrome.

In Thailand, researchers conducted a serological survey of workers who had been

involved in cleaning a pond in Khumuang, Buriram Province. Multivariable logistic regression indicated that wearing long pants or skirts was protective against leptospiral infection, while the presence of more than two wounds on the body was associated with infection.⁶⁷

In Malaysia isolation of *Leptospira* from black rats (*Rattus rattus*) was reported in 1928 by Fletcher.^{9,68} Many subsequent investigations have demonstrated a high prevalence of infection in humans in Malaysia,⁶⁹ with Robinson and Kennedy,⁷⁰ reporting 31 cases among British army personnel in Malaysia. From 1953 to 1955, 30 pathogenic leptospiral serovars were identified by Alexander,³¹ from both military personnel and civilians. Their studies demonstrated a high sero-prevalence in humans throughout Malaysia. The highest distribution was found in labourers working in rubber estates and those working with the sewage, drainage, forestry and town cleaning industries.³¹ In the 1950s and 1960's a comprehensive study of leptospirosis in Malaya was undertaken that included testing various mammals from a range of environments, as well as assessing occupational risks to humans. The results suggested that rats were the main maintenance hosts for leptospirosis, despite the presence of infection across many animal species. Over one hundred (104) strains were isolated and identified.⁷¹ Bahaman *et al*,³¹ conducted a cross-sectional serological survey of domestic animals in West Malaysia and found that approximately one quarter of the animals examined had agglutinating antibodies to *L. interrogans*. Cattle, buffaloes and pigs were all observed to have a high seroprevalence with temperate cattle breeds appearing more susceptible to infection than local breeds. A subsequent study found that the bacteriological seroprevalence in cattle and buffaloes was 14.4%.⁶⁸ A new serovar was isolated from a bovine kidney, while six other serovars were isolated for the first time from Malaysian cattle. Serovar hardjo was shown to be maintained in Malaysian cattle.³¹ Recently leptospirosis has been reported in detention centres for refugees in Malaysia.^{72,73} An outbreak at Juru Detention Centre was contained by the health authorities, but the death of six Burmese at an undisclosed detention centre in September 2009 has raised fresh concerns for several Malaysian NGOs. In July 2010, cases of leptospirosis were reported nation-wide and eight people who took part in a search and rescue operation in Lubok Yu, Maran, Pahang died from the disease, causing

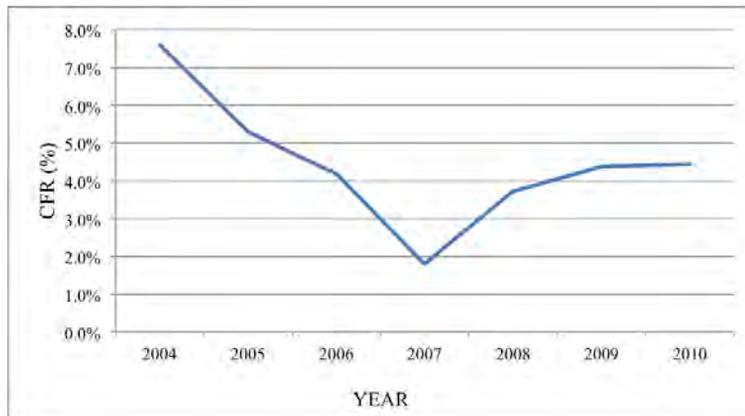


FIG. 1: Case Fatality Rate (CFR) of leptospirosis in Malaysia from 2004 to 2010.

the picnic spot to be closed to the public.^{74,75} Other cases were reported in Kedah during July 2011, with one fatality at Lata Bayu.⁷⁶ As recent as March 2012, a national service trainee died of suspected leptospirosis at Sungai Siput in Perak, Malaysia.⁷⁷ This resulted in a suspension of water based activities at all National Service Training camps.

According to data from the Ministry of Health the prevalence of leptospirosis increased dramatically from 2004 to 2009 (Table 1), however the number of deaths from leptospirosis did not change from 2004 to 2007, although it increased markedly to 47 and 62 deaths in 2008 and 2009, respectively. The highest numbers of cases were reported in the state of Perak during 2005, 2006, 2008 and 2009 with 71, 93, 289 and 280 cases, respectively (Table 1). In 2004, more cases³² were reported in Sarawak than in other years and in 2007 the highest numbers of

cases (184) were reported in Pahang. The states of Sarawak, Selangor and Terengganu showed a gradual increase in the number of cases over this period. No cases were reported in WP Labuan owing to its small geographical area with no forest habitat and the fact that it is surrounded by sea-water.

Case fatality rates (CFR) over the period from 2004 to 2009 varied from 1.8% to 7.6% (Figure 1) with an average of 4.44%. During this period a total of 5,267 cases of leptospirosis were reported nationwide with 234 known fatalities. Perak had the highest CFR for this period (6.81%), followed by Sarawak (6.42%) and Perlis (6.25%). Approximately one-fifth of all cases of leptospirosis for this period were documented in Perak state, of which 71 proved fatal (Figure 2). The almost threefold increase in the number of cases in 2007 and subsequently may be due to improved diagnostic techniques

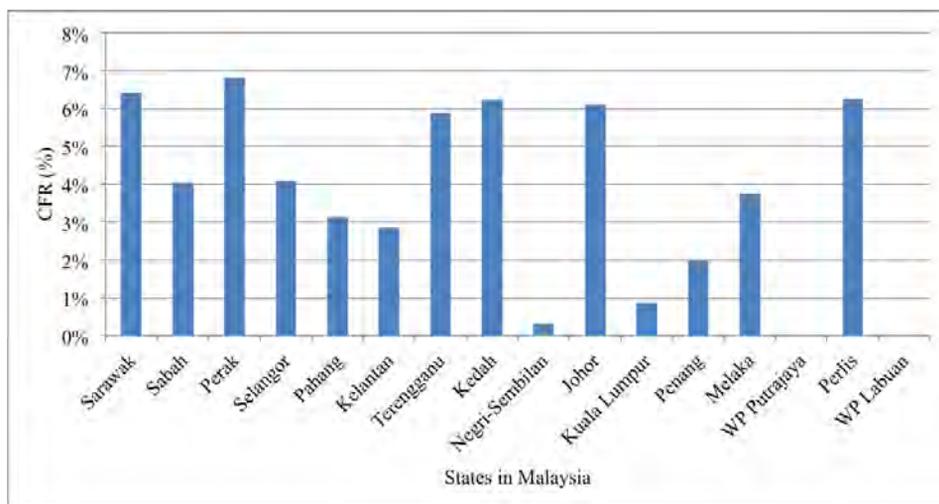


FIG. 2: Leptospirosis CFR in different Malaysian states from 2004 to 2009.

Table 1: Status of leptospirosis in humans in Malaysia from 2004 to 2009

| State | Year | | | | | | | | | | | | Total | |
|----------------|------------|-----------|------------|-----------|------------|-----------|-------------|-----------|-------------|-----------|-------------|-----------|-------------|------------|
| | 2004 | | 2005 | | 2006 | | 2007 | | 2008 | | 2009 | | | |
| | Cases | Deaths | Cases | Deaths | Cases | Deaths | Cases | Deaths | Cases | Deaths | Cases | Deaths | Cases | Deaths |
| Sarawak | 32 | 2 | 71 | 2 | 37 | 3 | 70 | 4 | 58 | 5 | 70 | 4 | 309 | 20 |
| Sabah | 13 | 1 | 12 | 1 | 19 | 0 | 35 | 1 | 34 | 2 | 35 | 1 | 148 | 6 |
| Perak | 29 | 4 | 71 | 4 | 93 | 9 | 280 | 19 | 289 | 16 | 280 | 19 | 1042 | 71 |
| Selangor | 16 | 5 | 20 | 3 | 37 | 0 | 208 | 7 | 97 | 2 | 208 | 7 | 586 | 24 |
| Pahang | 29 | 1 | 24 | 0 | 51 | 3 | 184 | 5 | 198 | 7 | 184 | 5 | 670 | 21 |
| Kelantan | 15 | 1 | 38 | 1 | 17 | 1 | 138 | 4 | 180 | 4 | 138 | 4 | 526 | 15 |
| Terengganu | 7 | 1 | 17 | 0 | 42 | 1 | 126 | 9 | 107 | 4 | 126 | 9 | 425 | 25 |
| Kedah | 15 | 1 | 27 | 0 | 31 | 0 | 106 | 9 | 52 | 2 | 106 | 9 | 337 | 21 |
| Negri-Sembilan | 27 | 1 | 41 | 0 | 24 | 0 | 91 | 0 | 59 | 0 | 91 | 0 | 333 | 1 |
| Johor | 30 | 1 | 29 | 6 | 31 | 2 | 59 | 3 | 87 | 3 | 59 | 3 | 295 | 18 |
| Kuala Lumpur | 31 | 0 | 20 | 1 | 27 | 1 | 54 | 0 | 45 | 0 | 54 | 0 | 231 | 2 |
| Penang | 9 | 0 | 25 | 1 | 28 | 0 | 32 | 0 | 25 | 2 | 32 | 0 | 151 | 3 |
| Melaka | 7 | 1 | 9 | 1 | 79 | 2 | 20 | 1 | 25 | 0 | 20 | 1 | 160 | 6 |
| WP Putrajaya | 1 | 0 | 1 | 0 | 7 | 0 | 14 | 0 | 1 | 0 | 14 | 0 | 38 | 0 |
| Perlis | 2 | 1 | 2 | 0 | 4 | 0 | 1 | 0 | 6 | 0 | 1 | 0 | 16 | 1 |
| WP Labuan | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 263 | 20 | 378 | 20 | 527 | 22 | 1418 | 62 | 1263 | 47 | 1418 | 62 | 5267 | 234 |

Sources: (3)

or a greater awareness of the disease. This may also have contributed to reducing the CFR from 2004. It is also possible that climate change in the last four years of this period played a part in influencing the pattern of infection, as outbreaks of leptospirosis usually follow after a major flood during monsoon seasons.

Status of leptospirosis in Sabah and Sarawak

As well as Peninsular Malaysia, scientific research has also focused on Malaysian Borneo. There have been reports of leptospirosis in humans in Sabah and Sarawak, notably an outbreak following the 2000 Eco-challenge event in Sabah.^{78,79} This highlighted the risks of eco-tourism and adventure travel, as most of the victims were western athletes. In the Eco-challenge event, participants competed in jungle trekking, swimming and kayaking in freshwater and caving. A team of researchers from the CDC Atlanta contacted 189 athletes from 27 countries following the outbreak.⁷⁹ Eighty athletes met their case definition, with 29 cases being hospitalized, although no fatalities were reported. Another study in Sabah revealed that swimming in the Segama River appeared to be a risk factor for infection and 20 athletes reported taking doxycycline prophylactically and this seemed to have a protective effect, with a preventive efficacy of 55%.

Studies carried out in Sabah reported a high seroprevalence (25.75%) in people living within the periphery of a national park, presumably due to exposure/contact with wild mammals.⁹ Leptospirosis has also been reported in a caver from the USA after he returned from Gunung Mulu National Park, in Sarawak.⁵² Recently researchers from Universiti Malaysia Sarawak (UNIMAS) and Sarawak Health Department have conducted research in the Rejang Basin area, Sarawak. They found that 30.6% of humans sampled were seropositive for leptospirosis and infection was associated with farming and/or water activities.⁸⁰ In Bakun in Sarawak, a Chinese national working on the hydro-electric dam project site was hospitalised with a serious condition suspected to be leptospirosis, and the subsequent death of 10 workers involved in the translocation of animals from the Bakun Hydroelectric Dam water catchment area to higher ground were speculated to be due to either leptospirosis or melioidosis.^{81,82}

In 2011, 186 cases of leptospirosis were

reported in humans in Sarawak including 13 deaths, compared with 49 cases in the previous year.⁸³ The CFR associated with this outbreak (6.9%) was higher than that in previous years. On the 12th December 2011, an outbreak occurred at RSAT Army camp at Penrissen Batu 8, Kuching, Sarawak with five army recruits showing symptoms of leptospirosis and the disease was subsequently confirmed serologically. The source of infection was identified as drinking and bathing activities in the small river near the camp.⁸⁴ The Kuching Divisional Health Office was notified of another suspected leptospiral outbreak on the 30th December 2011 by the Sarawak General Hospital. The incident involved two army recruits from Blok G10, Kem Semenggok, Kuching and both were serologically positive for leptospirosis.⁸⁵ The health authority of Sarawak confirmed a leptospirosis outbreak in the drains along the Tiong Hua Road in Sibu during June 2012.⁸⁶ In the state of Sarawak, the number of cases of leptospirosis was similar from 2004 to 2010, however in 2011 and 2012 there was a large increase in the number (Figure 3).

The four-fold spike in the number of cases in Sarawak is a valid cause for concern for health authorities in both Kuching and Kuala Lumpur. For 2011 alone, the CFR was 6.9%, above the six-year average of 6.42% (2004 and 2009). The number of cases (186) reported in 2011 alone was already equivalent to 60% of the combined total from 2004 to 2009. The record high of 271 documented cases the following year may be attributed to mandatory reporting procedures being implemented, or increased awareness of the symptoms of leptospirosis by medical practitioners. These also reflect on the living conditions of residents in various areas of Sarawak.

Conclusions

Over the years, leptospirosis has presented itself intermittently to the Malaysian health authorities and further research has consequently been conducted. The biggest issue for doctors has been the confusing nature of symptoms presented by affected humans. Since leptospirosis is a febrile illness and several tropical diseases manifest in their early stages with fever, it is easy to misdiagnose the disease unless further laboratory testing is undertaken. This can result in delayed commencement of appropriate treatment (single dose doxycycline therapy). Fortunately with advances in medical technology,

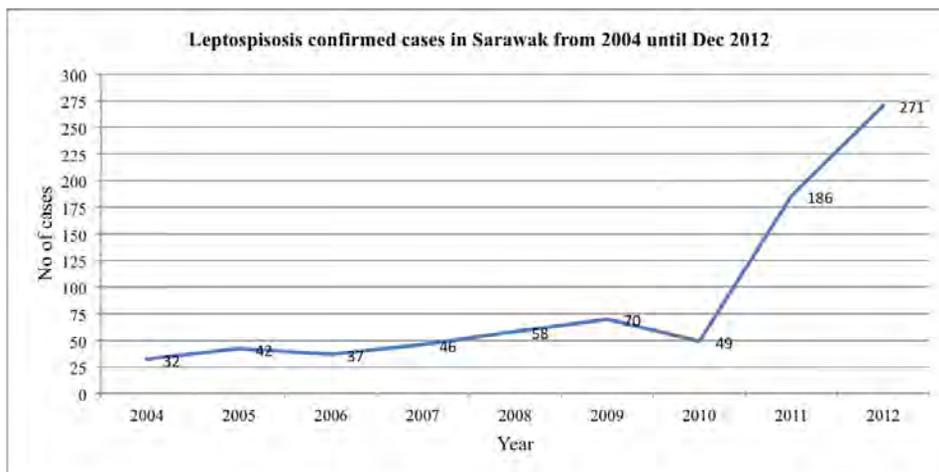


FIG. 3: Number of confirmed cases of leptospirosis in humans in Sarawak

diagnosis of leptospirosis has become more accurate. Currently there is much research being undertaken on leptospirosis, however due to poor coordination of this research the actual incidence of disease is not truly reflected in Malaysia. There is a need for cooperation between researchers from veterinary and medical backgrounds to determine the real status of leptospirosis. Loss of biodiversity is a possible outcome of this disease as wildlife comes under constant threat and increasing pressure to survive. Emerging zoonotic infectious diseases represent a growing threat and danger to wildlife as well as humans. Real-time surveillance through integrated human, veterinary and wildlife disease systems will reduce the time taken to recognise disease and implement suitable disease control practices.

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