Prevalence of leptospiral DNA among wild rodents from a selected area in Beguk Dam Labis, Segamat, Johor, Malaysia

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Abstract

Leptospirosis is an emerging infectious disease. The differential diagnosis of leptospirosis is difficult due to the varied and often “flu like” symptoms which may result in a missed or delayed diagnosis. Leptospira is the aetiological agent of leptospirosis, a bacterial zoonosis with worldwide distribution. There are over 230 known serovars in the genus Leptospira. The true prevalence of leptospirosis in Malaysia is unknown or underestimated. Our goal was to determine the prevalence for Leptospira infection in rodents in a selected area in Beguk Dam Labis, Segamat, Johor. A study was carried out on 69 serum samples of trapped wild rodents. DNA was extracted from the sera using Leptospira PCR kit (Shanghai ZJ Bio-Tech Co., Ltd). Of 69 rodent serum samples tested by PCR, 9 (13%) showed positive results. In this study we found that (13%) of wild rodents caught in Beguk Dam Labis were infected by Leptospira.

Keywords: Leptospirosis, wild rodents, PCR, serum

INTRODUCTION

Leptospira is the aetiological agent of leptospirosis, a bacterial zoonosis with worldwide distribution. Leptospirosis is caused by Leptospira sp, a spirochete aerobic bacterium, gram-negative, with spiral morphology. It is an important global disease with public and animal health implications. Reported cases of leptospirosis have increased lately. An outbreak of leptospiral infection was reported among athletes participating in the Eco-Challenge-Sabah 2000 held in Malaysian Borneo. The infection was reported to be associated with water-related activities. Investigations of large outbreaks would be greatly enhanced by the availability of rapid and sensitive diagnostic assays which can confirm the diagnosis early in the clinical illness. There are over 230 known serovars in the genus Leptospira. The disease is maintained in nature by chronic renal infection of carrier mammals, which excrete the organism in their urine. A study conducted in 2009 in Malaysia indicated that Leptospira serovars were prevalent in the Malaysian rat population and could be a source of infection to humans. Diagnosis of leptospirosis is usually accomplished retrospectively by serology, because culture requires both special media and incubation for several weeks. Serological diagnosis by microscopical agglutination test invariably requires testing of acute and convalescent sera, since agglutinating antibodies often are not detectable during the acute illness. IgM antibodies become detectable 5–7 days after the onset of symptoms, and the use of IgM-ELISA assays for presumptive diagnosis has been evaluated in numerous populations. Since leptospires are difficult to culture, several PCR methods have been used to facilitate early diagnosis. A number of PCR assays for leptospiral DNA have been described, but only two have been evaluated in clinical studies and used extensively for diagnosis. PCR assays which could detect all pathogenical and non-pathogenical leptospires in clinical samples were also described. More recently, a real-time PCR was developed using TaqManchemistry (which targets an 87bp section of the16S rRNA gene of Leptospira spp. Our goal was to determine the frequency for Leptospira infection in rodents in a selected area in Beguk Dam Labis, Johor.
MATERIALS AND METHODS

Origin of rodents
Sixty-nine wild rodents were trapped from Labis, Johor. Serum samples were obtained from the rodents.

Genomic DNA extraction and PCR
Briefly the methods were as follows: DNA extraction and PCR was performed using Leptospira PCR kit (Shanghai Z Biotech). 10 μl of serum was pipetted to a 0.5 ml tube, 50μl DNA extraction buffer was added. The tube was vortexed for 10 seconds followed by incubation at 100 °C for 10 minutes. The tube was then centrifuged at 13000 rpm for 10 minutes. Supernatant containing genomic DNA was used for PCR template. PCR program was as follows: 94°C for 2 minutes, followed by 35 cycles at 93°C for 15 sec, 55°C for 30 sec, 72°C for 30 sec.

RESULTS

Results of PCR using Leptospira PCR kit (Shanghai ZJ Bio-Tech Co., Ltd) are as shown in Fig. 1. Of 69 serum rodents tested, 9 (13%) were PCR positive, generating a 285 base pair product. Some of the positive samples demonstrated weak bands probably due to quality of the DNA extracted from serum samples. This may be due to the quality of the DNA extracted from serum samples.

DISCUSSION

A rapid, simple and accurate method is needed for the diagnosis of leptospirosis. Direct demonstration of Leptospira in clinical samples such as blood, urine and CSF performed by bacterial culture takes too long time and is insensitive.12 Serum antibody detection, thus, served as an indirect alternative means of leptospirosis diagnosis. MAT is one of the commonly used antibody detection assays.13 MAT is insensitive during the early phase of infection and requires a large battery of living Leptospira spp. of various serogroups and serovars which are laborious and costly.12 Other simpler antibody detection methods have been developed, such as indirect immunoﬂuorescent assay, IgM ELISA and IgM dipstick. Sensitivity of these assays, however, is still limited by the Leptospira spp. used for preparing the antigens. False negative results may occur if the infecting Leptospira spp. does not match the Leptospira spp. used as antigen in the assays. Alternatively, false positive results may be obtained by antibodies in serum of patients previously infected by unrecognized Leptospira or exposure to antigenically related organisms especially in leptospirosis endemic areas.14

In this study, PCR technique was applied to detect the presence of leptospiral DNA in sera samples of wild rodents. All of the rodents tested were species of Rattus tiomanicus. Leptospirosis is reported to be more prevalent

![Ethidium bromide stained agarose gel electrophoresis of PCR amplified DNA extracted from serum using Leptospira PCR kit, Shanghai ZJ Bio-Tech Co.](image-url)

**FIG. 1:** Ethidium bromide stained agarose gel electrophoresis of PCR amplified DNA extracted from serum using Leptospira PCR kit, Shanghai ZJ Bio-Tech Co. Lane 1: DNA marker (100 bp ladder). Lane 2: Leptospira interrogans serovar bataviae (positive control). Lane 3 - Lane 13: rodents’ serum samples. The positive sera are lane 3 (L4345), lane 4 (L350), lane 5 (L55), lane 6 (L357), lane 7 (L361), lane 8 (L366), lane 10 (L378), lane 11 (L381), lane 12 (L384) and lane 13 (L389).
in tropical and subtropical countries. This study showed that 13% of rodents tested were positive. The relatively high infection rate of 13% among rodents could represent a hazard. This area was hit by floods in 2008, indicating it as a flood-prone area. Leptospirosis can be transmitted to both humans and animals by direct contact with the urine of infected rodents in contaminated flood water. Large clusters of cases have been reported in Central and South America following flooding as a result of El Niño-related excess rainfall. However, the occurrence of large outbreaks of leptospirosis following severe floods is not a new phenomenon and is not restricted to tropical regions. The results of our study suggest that serum samples serve as good source of genomic DNA for PCR amplification for detection of Leptospira in wild rodents. This method could probably be recommended for detection of Leptospira in other potential host animals.

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