Malaysian J Pathol 2012; 34(2) : 157 – 159

BRIEF COMMUNICATION

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

I Latifah *PhD,* MS Rahmat*, KB Hayarti *MIMLS,* S Paramasvaran,** MR Azizah, F Imran *MD**** and Y Normaznah *PhD*

*Biotechnology Unit, *Biomedical Museum Unit, **Epidemiology & Biostatistics Unit, ***and Bacteriology Unit MRRC, Institute for Medical Research, Kuala Lumpur, Malaysia.*

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.[1] It is an important global disease with public and animal health implications.[2] Reported cases of leptospirosis has increased lately.[3] 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.[3] 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.[3] 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.[4] 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.[5] Diagnosis of leptospirosis is usually accomplished retrospectively by serology, because culture requires both special media and incubation for several weeks.[6] 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.[7] Since leptospires are difficult to culture, several PCR methods have been used to facilitate early diagnosis.[8] 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.[9] PCR assays which could detect all pathogenical and non-pathogenical leptospires in clinical samples were also described.[10] More recently, a real-time PCR was developed using TaqManchemistry (which targets an 87bp section of the16S rRNA gene of *Leptospira* spp.[11] Our goal was to determine the frequency for *Leptospira* infection in rodents in a selected area in Beguk Dam Labis, Johor.

Address for correspondence and reprint requests: Dr. Latifah Ibrahim, Biotechnology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia. Tel: +603-26162438. Email: latifah@imr.gov.my

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 *Leptospra* spp. of various serogroups and serovars which are laborious and costly.[12] Other simpler antibody detection methods have been developed, such as indirect immunoflorescent 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 *Rattustiomanicus*. Leptospirosis is reported to be more prevalent

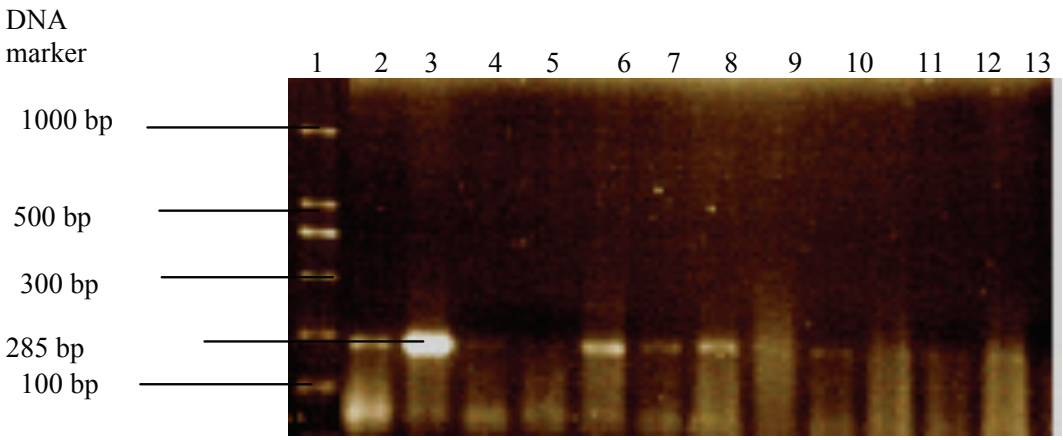

FIG. 1: Ethidium bromide stained agarose gel electrophoresis of PCR amplified DNA extracted from serum using *Leptospira* PCR kit, Shanghai ZJ Bio-Tech Co.
Lane 1: DNA marker (100 bp ladder). Lane 2: *Leptospira interrogans* serovar *bataviae* (positive control). Lane 3 - Lane 13: rodents' serum samples. 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.[15, 16] However, the occurrence of large outbreaks of leptospirosis following severe floods is not a new phenomenon and is not restricted to tropical regions.[17, 18] 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 *Leptopsira* in other potential host animals.

ACKNOWLEDGEMENTS

We wish to thank the Director-General of Health, Ministry of Health, Malaysia for his permission to publish this paper. We also thank the Director of the Institute for Medical Research for support and encouragement to publish this paper. This project was financially supported by the National Institutes of Health Malaysia grant to the Institute for Medical Research grant (Grant no: JPP-IMR33/06)

REFERENCES

1. Bharti AR, Nally JE, Ricaldi JN, *et al*. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis. 2003; 3(12): 757-71.
2. McBride AJ, Athanazio DA, Reis MG, Ko AI. Leptospirosis. Curr Opin Infect Dis. 2005; 18(5): 376-86.
3. Sejvar J, Bancroft E, Winthrop K, *et al*. Leptospirosis in "Eco-Challenge" athletes, Malaysian Borneo, 2000. Emerg Infect Dis. 2003; 9(6): 702-7.
4. Faine S, Adler B, Bolin C, Perolat P. Leptospira and Leptospirosis. 2nd ed. Melbourne: MediSci; 1999.
5. Mohamed-Hassan SN, Bahaman AR, Mutalib AR, Khairani-Bejo S. Serological prevalence of leptospiral infection in wild rats at the National Service Training Centres in Kelantan and Terengganu. Trop Biomed. 2010; 27(1): 30-2.
6. Levett PN. Leptospira and Leptonema. In: Murray PR, Baron EJ, Jorgensen JH, editors. Manual of clinical microbiology. Washington DC: American Society of Microbiology; 2003. p. 929-36.
7. Bajani MD, Ashford DA, Bragg SL, *et al*. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. J Clin Microbiol. 2003; 41(2): 803-9.
8. Brown PD, Gravekamp C, Carrington DG, *et al.* Evaluation of the polymerase chain reaction for early diagnosis of leptospirosis. J Med Microbiol. 1995; 43(2): 110-4.
9. Merien F, Baranton G, Perolat P. Comparison of polymerase chain reaction with microagglutination test and culture for diagnosis of leptospirosis. J Infect Dis. 1995; 172(1): 281-5.
10. Gravekamp C, Van de Kemp H, Franzen M, *et al*. Detection of seven species of pathogenic leptospires by PCR using two sets of primers. J Gen Microbiol. 1993; 139(8): 1691-700.
11. Smythe LD, Smith IL, Smith GA, *et al*. A quantitative PCR (TaqMan) assay for pathogenic Leptospira spp. BMC Infect Dis. 2002; 2: 13.
12. Ellinghausen HC, Jr. Growth, cultural characteristics, and antibacterial sensitivity of Leptospira interrogans serovar hardjo. Cornell Vet. 1983; 73(3): 225-39.
13. Appassakij H, Silpapojakul K, Wansit R, Woodtayakorn J. Evaluation of the immunofluorescent antibody test for the diagnosis of human leptospirosis. Am J Trop Med Hyg. 1995; 52(4): 340-3.
14. Levett PN. Leptospirosis. Clin Microbiol Rev. 2001; 14(2): 296-326.
15. Ko AI, Galvao Reis M, Ribeiro Dourado CM, Johnson WD, Jr., Riley LW. Urban epidemic of severe leptospirosis in Brazil. Salvador Leptospirosis Study Group. Lancet 1999; 354(9181): 820-5.
16. Zaki SR, Shieh WJ. Leptospirosis associated with outbreak of acute febrile illness and pulmonary haemorrhage, Nicaragua, 1995. The Epidemic Working Group at Ministry of Health in Nicaragua. Lancet. 1996; 347(9000): 535-6.
17. Park SK, Lee SH, Rhee YK, *et al*. Leptospirosis in Chonbuk Province of Korea in 1987: a study of 93 patients. Am J Trop Med Hyg. 1989; 41(3): 345-51.
18. French JG, Holt KW. Floods. In: Gregg MB, editor. The public health consequences of disasters. Atlanta: US Department of Health and Human Services, Public Health Service, CDC; 1989. p. 69-78.