

Leptospires in field Rats in and around the laboratory animal facilities of Bangalore, India

Vinodkumar, G., Rajeshwari, Y. B., Shivaraj¹, U. Krishnamoorthy, Ansar Kamran²

Department of Livestock production and management,
Veterinary college, KVAFSU, Bangalore-24.

1. Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore, India.

2. Department of Clinical Medicine, Veterinary college, KVAFSU, Bangalore-24.

* Corresponding author email: vindy686@gmail.com

Received: 09-12-2010, Accepted: 04-02-2011, Published Online: 16-07-2011

doi: 10.5455/vetworld.2011.410-412

Abstract

The present study was undertaken to determine the prevalence of leptospires in field rats in and around laboratory animal facilities in Bangalore. 34 rats were trapped alive in and around the laboratory animal facilities in Bangalore. Urine and serum samples from these field rats were collected. Serum samples were tested for anti-leptospiral antibodies by microscopic agglutination test, while urine samples were subjected for dark field microscopy and polymerase chain reaction to detect the presence of leptospiral antigens. Serology revealed the presence of anti-leptospiral antibodies in 19 (61.29 percent) field rats and dark field microscopy revealed the presence of leptospiral antigens in 3 (8.82 percent) and 6 (17.65 percent) of urine samples of these field rats. Among the serovars, *Icterohaemorrhagiae* was predominant followed by *Autumnalis* and *Pyrogens*. Serology dark field microscopy and polymerase chain reaction reveals that field rats are major natural carriers and shedders of leptospires.

Key words: Leptospirs, Zoonosis, Laboratory Animal, Rodent, Antibody, Antigen, Urine, Dark field microscopy, PCR.

Introduction

Leptospirosis is wide spread zoonosis caused by pathogenic spirochetes of the genus *Leptospira*. It assumes zoonotic importance since rodents and certain species of domestic animals such as dogs, pigs and cattle are known to be natural carriers of various serovars thus forming infection reservoirs in transmission of infection to other animals and man, especially professionally vulnerable group such as veterinarians, animal care takers. Certain vertebrate animal species have a commensal relationship with leptospires in which they are the natural hosts for pathogenic leptospires that live in their kidneys. Such leptospires do little or no detectable harm to these hosts but they maintain the infection and are therefore known as natural maintenance hosts. If other animals that are not natural maintenance hosts (including humans) are infected by the same pathogenic leptospires, they often become ill. In addition, if a maintenance host for a particular leptospire is infected with another serovar it may develop symptoms and signs of Leptospirosis (WHO 2003).

Rodents are carriers of leptospires throughout the world and are important reservoirs of infection for man and domestic animals (Twigg *et al.*, 1969; Hathaway and Blackmore, 1981). There are three

species of rodents that are distributed worldwide and are commonly associated with leptospiral infection: *Mus musculus* (house mouse), *Rattus norvegicus* (brown rat) and *Rattus rattus* (black rat). All three species belong to the order Rodentia, family Muridae, subfamily Murinae. In rodents, especially the common rat (*Rattus norvegicus*) and other reservoir species, the organisms persist indefinitely in the convoluted tubules of the kidney without causing apparent disease, and are shed into the urine in massive numbers (Dutta and Christopher *et al.*, 2005). *Rattus rattus wroughtoni hinton*, *Rattus rattus rufesens*, *Bandicota indica* and *Bandicota bengalenses* are the rodent species involved in spreading leptospirosis to human and live-stock in India (Gangadhar *et al.*, 2005). With this in view the present study has been undertaken with the objectives to record the prevalence of leptospirosis in field rats in and around laboratory animal facilities in Bangalore.

Materials and Methods

Present study was undertaken at veterinary college Bangalore. 34 urine and 31 serum samples from field rats trapped alive in and around five different laboratory animal colonies in Bangalore were collected. Field rats were anesthetized by means

Table-1. Panel of leptospiral reference strains used for microscopic agglutination test.

S.No	Serogroup	Serovar
1	<i>Icterohaemorrhagiae</i>	<i>Icterohaemorrhagiae</i>
2	<i>Autumnalis</i>	<i>Autumnalis</i>
3	<i>Pomona</i>	<i>Pomona</i>
4	<i>Pyrogens</i>	<i>Pyrogens</i>

of Diethyl Ether inhalation. Blood samples for serology were collected by cardiac puncture. The abdomen was then opened and urine was collected from bladder under sterile conditions using 2 ml syringe. Collected urine was then transferred to 5 ml test tube filled with 2 ml of Phosphate Buffer Saline (PBS). Cold chain was maintained while transporting these samples. The tubes containing blood samples were kept in a slanting position for 1 to 2 hours, serum was separated from the clotted blood by centrifuging at 2000 rpm for 2 min and transferred to 2 ml screw-capped plastic vials and were stored at - 20°C until tested.

Microscopic agglutination test: A panel of four serovars was used for the MAT (Table 1). MAT was conducted as per the OIE (2004) in 96 well U bottom titration plates with initial dilution of 1:100 upto 1:1280. the highest dilution of serum showing 50 per cent reduction in number of free leptospire was considered as the end-titer.

Dark field microscopy: Urine samples collected in phosphate buffer saline were centrifuged at 12000 rpm and the sediment suspended in small volume of PBS to form wet mount preparation and was examined under dark field microscope.

Polymerase chain reaction: PCR assay was carried out as per the method described by Gravekap *et al.*, (1993) with slight modifications. The urine samples collected in phosphate buffer saline were centrifuged at 10,000 rpm for 30 minutes. The pellets were resuspended in 0.1 ml distilled water and washed two more times with distilled water. The final pellets were resuspended in 20 µl of distilled water. The pellet samples were denatured at 96°C for 10 minutes and snap cooled in icebox. This was used as a DNA template. Amplification of DNA was performed in a total volume of 25 µl. The reaction mixture contained 12.5 µl of master mix, 9.5 µl of distilled water, 1 µl of each primer G1 and G2 (Primer G1: 5'- CTG AAT CGC TGT ATAAA GT-3', Primer G 2:5'- GGAAA CAA ATG CTC GGA AG-3') and 2 µl of DNA template. The samples were placed in automatic PCR processor (M J Research minicycler). One amplification cycle consisted of annealing of primers for 1 min at 55°C, elongation for 2 min at 72°C, and

denaturation for 5 min at 94°C. The number of cycles 33 were used. The last elongation step was extended to 6 min. After amplification a 10 µl portion of each sample was subjected to electrophoresis on a 1.3% agarose gel at 100 V for 20 to 30 minutes until the tracking dye migrated more than two third of the length of the gel tray in the buffer and the results were documented in a gel documentation system (Biorad).

Results

Seroprevalence of leptospiral antibodies by MAT among field rodents trapped from within and/or around the premises of different laboratory animal facilities revealed 19 (61.29 per cent) samples were positive by agglutinating with one or more of the four leptospira antigens tested, out of 31 sera tested. The serovar distribution among laboratory rats in the descending order was *Icterohaemorrhagiae* (11; 57.89 per cent), *Autumnalis* (5; 26.32 per cent), *Pyrogens* (3; 15.78 per cent). The positive titres in field rats were 1:100 (5.26 per cent), 1:200 (5.26 per cent), 1:400 (15.79 per cent), 1:800 (5.26 per cent), 1:1600 (31.58 per cent), 1:3200 (26.32 per cent), 1:6400 (5.26 per cent) and 1:12800 (5.26 per cent).

Dark Field Microscopy (DFM) detected leptospire in 3 (8.82 per cent) field rat urine samples of the total 34 samples. Where as PCR detected leptospiral DNA in 6 (17.65 per cent) field rat urine samples of the total 31 samples.

Discussion

The overall seropositivity of field rats in this study was 61.29 per cent. Earlier reports from India have shown seropositivity at different levels of 88 per cent (Saadi, 1976), 7.1 per cent (Sharma *et al.*, 2003), 58 per cent (Priya *et al.*, 2008). The magnitude of the seropositivity alters from region to region largely attributed to climatic and environmental conditions (WHO 2003). Serovar *Icterohaemorrhagiae* was predominant in these field rats. But in earlier reports the predominant serovar was *autumnalis* in and around Madurai (Priya *et al.*, 2008) and also in and around Bangalore (Syed, 1978). 57.9 per cent were having titre of 1600 to 3200, where as above 3200 was 10.52 and lower than 1600 was 31.57. Titre above 800

recorded was predominantly against Serovar *Icterohaemorrhagiae*.

Of the 34 urine samples of field rats, only 3 (8.82 per cent) leptospires could be detected through DFM. Only 6 (17.65 per cent) samples out of 34 urine samples were found positive for the presence of leptospiral antigen by PCR. Less positivity of PCR and DFM may be due to the less concentration of leptospiral organisms in the given samples, as minimum of 10^4 leptospores/ml of urine is required for detection (Paula *et al.*, 2004). It is believed that once a rodent is infected with Leptospires it acts as carrier and irregular shedding of leptospires for the rest of its life, perpetuating the dissemination of leptospires. All the rodents showing seropositivity by MAT may not be shedding the organisms on the day the samples were collected.

Acknowledgements

The authors would like to thank The Head, Department of Livestock Production and Management, Veterinary College Bangalore, KVAFS University for providing facilities.

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