

Detection of Leptospiral Antibodies in the Sera of Captive Elephants

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Abstract

In the present study, serum samples were collected from 51 captive elephants kept in the three different forest ranges (19-Bandipur, 12-Shimogga, and 20-Mudhumalai). The samples were subjected to screening for leptospirosis by microscopic agglutination test (MAT). It was found that out of the 51 samples seven elephant sera (13.72%) showed antibody titres against two serovars of *Leptospira interrogans* (*L. australis* and *L. canicola*) by MAT indicating the presence of infection or due to the past exposure of captive elephants to leptospiral antigens.

Keywords: Elephants, Leptospirosis, MAT, Forest, Captive, Antibodies.

Introduction

Leptospirosis is an infectious disease of man and animals caused by antigenically distinct members of the genus *Leptospira* with a broad spectrum of host range including the wild life. The public health aspects of the disease acquire more significance especially in countries like India due to the close association between man and animals. The pathogenic leptospires, *Leptospira interrogans* contains about 223 serovars organized into 23 serogroups (Venkatesha, M.D., 1997). Differentiation of these leptospires is dependent upon microscopic agglutination test (MAT) with serogroup and serovar specific hyperimmune sera. Upadhye, *et al.* (1979) detected antibodies against *Leptospira valbuzzi* and *Leptospira pyrogenes* in the sera of elephants. Narayana Bhatt *et al.* (1998) detected antibodies against *L. Pomona*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. hebdomadis*, *L. hardjo*, *L. canicola*. The complexity of epidemiology of leptospires in wildlife has not been fully studied in India. Keeping this point in view in the present study an attempt was made to screen the serum samples of captive elephants by using microscopic agglutination test (MAT) to study the seroprevalence of leptospires in captive elephants.

MAT is one of the important serological tests used for the diagnosis of leptospirosis. It is highly sensitive and specific test and considered as reference test as

cited in OIE list for diagnosis of Leptospirosis. The antibody may be due to any of the large number of different serovars. Hence it is necessary to use a series of different serovars. Since leptospiral antibodies may be present for a considerable period of time after infection in the serum, so the sero-reactivity of serum samples by MAT may indicate the present or past exposure to leptospiral antigens. A MAT titre of 1:100 or above is taken as positive as per WHO manual for leptospirosis.

Materials and Methods

A total of 51 Serum samples were collected from the ear vein of captive elephants kept in elephant camps in different forest ranges (19-Bandipur, 12-Shimogga, and 20-Mudhumalai). The serum samples were inactivated at 56°C for 30mins before the test is conducted. In the present study eight serovars of *Leptospira interrogans* (*L. australis*, *L. autumnalis*, *L. canicola*, *L. icterohaemorrhagiae*, *L. pyrogenes*, *L. grippotyphosa*, *L. javanica* and *L. pomona*) maintained in the EMJH medium (Difco) by the Diagnostic Bacteriology and Mycology, IAH & VB, Hebbal, Bangalore were used. Sera were tested for the presence of specific antibodies against leptospira by Microscopic agglutination test (MAT) as per Merien, *et al.* (1995) with slight modification.

The test was carried out using actively grown culture of leptospiral serovars, which were obtained after inoculating into EMJH medium and allowing to

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Table-1. Details of Leptospiral Antibodies in the Sera of Captive Elephants by MAT

<i>Leptospira interrogans</i> serovar-strain	No. of sera tested	Titrewise Distribution			
		1:100	1:200	1:400	1:800
<i>Leptospira interrogans</i> serovar <i>australis</i>	51	3	-	-	-
<i>Leptospira interrogans</i> serovar <i>australis</i> and <i>canicola</i>	51	2	-	-	-
<i>Leptospira interrogans</i> serovar <i>canicola</i>	51	2	-	-	-
<i>Leptospira interrogans</i> serovar <i>pyrogen</i>	51	-	-	-	-
<i>Leptospira interrogans</i> serovar <i>grippityphosa</i>	51	-	-	-	-
<i>Leptospira interrogans</i> serovar <i>javanica</i>	51	-	-	-	-
<i>Leptospira interrogans</i> serovar <i>pomona</i>	51	-	-	-	-
<i>Leptospira interrogans</i> serovar <i>autumnalis</i>	51	-	-	-	-
<i>Leptospira interrogans</i> serovar <i>icterohaemorrhagiae</i>	51	-	-	-	-
Total	51	7	-	-	-

grow for 7-8days at room temperature. The serum samples were diluted to 1:10 dilution using phosphate buffer saline (PBS). A 50µl volume of PBS was added in all the 12 wells of the flat bottom 96 wells microtitre plates. Then the diluted serum sample was added in the first well and a serial dilution was made from the first to the 12th well of the row i.e. 1:50, 1:100, 1:200, 1:400,....., up to 1:102400 dilution using sterile PBS (pH 7.4). To this an equal volume of grown liquid culture was added and the micro titer plates were covered and incubated at room temperature for 2 hrs. After the incubation period of 2hrs a loopful of each dilution was examined for agglutination reaction under dark field microscope. The end point is the highest dilution showing 50% agglutination or above. A MAT titre of 1:100 or above is considered as positive for the infection against that serovar.

Results and Discussion

Out of the total 51 samples screened for antibodies by MAT seven elephants sera (13.729%) showed an antibody titres of 1:100 to two serovars of *L. australis* and *L. canicola*. Three serum samples showed antibodies against *L. australis* alone, two samples showed antibodies against *L. australis* and *L. canicola* and two samples showed antibodies against *L. canicola*. (Table 1).

Leptospiral infection has been recorded more frequently in animals and men coming in contact with infected water source, sugarcane or rice fields

(Rajashekar, M., 1968). Although direct transmission through skin abrasions occurs frequently, it is evident from the habitat of Elephants that water bodies play an important role in its survival either for drinking purpose or invariably for wallowing. Subsequently the elephants might have been exposed to the antigen through contaminated urine of canines, rodents or other infected animals shedding the antigen in the water source. Presence of antibodies against *Leptospira canicola* in elephant sera was already reported by Narayana Bhatt *et al.*, (1998) which might be due to the wild canines or other animals and the presence of antibodies against *L. australis* points towards the cattle grazing in nearby areas of the captive elephants.

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