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STUDIES ON PREVALENCE OF LEPTOSPIROSIS IN WILD ANIMALS

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Abstract: A total of 48 blood samples were collected from jackals, Hyenas, Deer, Lion and Elephants showing pyrexia and haemoglobineuria, bloody diarrhoea and jaundice. Blood samples were initially screened for Leptospira under dark field microscopy and attempted isolation in EMJH liquid media. The sera samples separated from blood were subjected for MAT (Microscopic Agglutination Test) for screening against Leptospiral antibodies. Out of 48 samples 15 were found positive for Leptospiral antibodies with positivity of 31.25%. The serovar analysis indicated the prevalence of L.*javanica*, L.*icterohaemorragica* and L.*hardjo*. Further, the biochemical analysis revealed the elevated levels of total serum bilirubin (>0.55ng/dl), SGOT (>135 IU/lt) and SGPT (>45dl/lt). The infected animals were treated with antibiotics, fluid supportive therapy for a period of two days and recovery was observed.
Keywords: Wild animals, seroprevalence, MAT, L.*javanica*, L.*icterohaemorragica* and L.*hardjo*, Bio-chemical analysis.

Introduction

Leptospira has emerged important zoonotic disease globally (Assimina et al 2003). It is caused by a spirochete, order spirochetales, family Leptospiracea and genus Leptospira. The disease assumes an important epidemiological role because it affects domestic and wild species. In places such as zoos and parks several species must live in restricted areas. This condition can disseminate numerous infectous agents that may cause wild variey of zoonotic diseases (Siemering et al 1993). So far, no information about the prevalence in wild animals in Andhra Pradesh and there was limited study on the complexity of epidemiology of Leptospira in wild life in India. Hence, the present study was aimed to study the prevalence of Leptospira in wild species.

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Material and Methods

Sample collection:

Leptospirosis was suspected in wild animals of SV Zoological park, Tirupati, Andhra Pradesh during period September 2009 – January 2010. A total of 48 blood samples were collected from Jackals, Hyenas, Deer, Lion and Elephants showing the symptoms of pyrexia, bloody diarrhea, dull and depressed and in Jackals, anorexia and jaundice suspected for Leptospirosis. All the samples were subjected for dark field micoscopic examination, cultural examination and serology by MAT. Further, the serum samples were also subjected by biochemical analysis for the estimation of total serum bilirubin, SGOT and SGPT.

Dark field microscopy:

All the blood samples collected were initially screened for the presence of Leptospires using dark field microscopy.

Isolation in EMJH media:

Ellinghon Macclough Johnson and Harris (EMJH) media was prepared according to Hohnson and Harris (1967) with slight modifications. All the 48 blood samples were inoculated into EMJH liquid media and incubated at 30^0C $\pm$ 1^0C in BOD incubator. The samples were screened for the presence of Leptospira at weekly intervals under the dark field microscope.

Microscopic agglutination test (MAT):

All the 48 sera samples from different species of wild animals were subjected for MAT. The MAT which was standardized at Leptospira diagnostic laboratory, C.V.SC, Tirupati, Andhra Pradesh was used to screen the sera samples against Leptospiral antibodies. Leptospira reference strains (Table: 1) were obtained from regional Medical Research Center (RMRC), Portblair, Andaman and Nicobar Islands. The battery of antigens used for MAT were maintained in EMJH liquid media.

Preparation of antigens for MAT:

The reference serovar strains were grown in EMJH liquid media about 5ml of EMJH liquid media was inoculated each with 0.5ml of fresh stock culture of reference serovar strains. Then the inoculated cultures with density of 2-3 X 10^9 organisms per ml were used as antigens.

A MAT titer of 1:80 and above which was standardized at Leptospira diagnostic laboratory, C.V.SC, Tirupati is considered as positive for the infection against that particular serovar.

Biochemical analysis:

The 48 sera samples were inactivated at 56^0C for 30 min and subjected for biochemical analysis for the estimation of total bilirubin, SGOT and SGPT using San diagnostics Pvt Ltd, India for further confirmation of Leptospira.

Results, Discussion and Treatment:

All the 48 blood samples collected from different wild species were found negative for Leptospira on dark field microscopic examination. During the study no organisms were recovered on isolation in EMJH liquid media. The success of isolation depends on time of collection, type of material and interval between collection and processing of samples (Theirmann 1984; OIE 2008 and Venkatesha 2009).

MAT is the gold standard test in diagnosis of Leptospira as recommended by OIE (2008). Out of 48 serum samples screened against Leptospira antibodies using MAT, only 15 sera samples were found positive showing seropositivity of 31.25% (Table: 2). The reactivity of serum samples by MAT may indicate the presence of post exposure to Leptospiral antigens. With respect to serovar analysis revealed the prevalence of L.*javanica* (35.3%), L. *icterohaemorragica* (35.2%) and L.*hardjo* (29.4%) in the serum samples collected from Jackals, Hyenas, Deer and Elephants. Similarly Narayana Bhat et al 1998, Shivaraj et al 2009 and Lilenbiun et al 2009 also reported the prevalence of Leptospira in wild animals. In Lions, no reactivity of reference serovar strains was noticed during the study. The sero prevalence from Jackals, Hyenas and Deer was reported and this might be due to contamination of soil and water with urine and tissues of infected animals. Further, on observation it was found that the particular area was densely populated with rodents; reservoir host for the transmission of Leptospira.

The important cause for the prevalence of Leptospira in Elephants might be due to their wallowing habit and contamination of water bodies with urine of infected animals and rodents. The occurrence of L.*hardjo* in Deer and Elephants might be due to acquisition of infection from near by grazing cattle of their captivity, the reservoir host for L.*hardjo* (Narayana Bhat et al 1998).

1st day schedule:

Intacef – 0.5g I/V

DNS – 600ml I/V

RL – 200ml I/V

Melonex – 2ml I/M

Toxol – 2ml I/M

2nd day onwards:

Oxy tetracycline – 3g/ animal mixed in chicken

LIV 52 – 7ml/ animal mixed in milk.

This treatment given upto 20 days.

Biochemical analysis of the respective sera samples of wild animals on estimation shown elevated levels of total serum bilirubin (>0.55ng/dl), SGOT (>135 IU/lt) and SGPT (>45dl/lt) indicating the liver damage which is further confirmation of Leptospirosis biochemically.

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Table 1: Reference strains of Leptospires used in the present study

S.No	Serogroup	Serovar	Strain
1	L. autumnalis	Rachmati	Rachmat
2	L. icterohaemorragica	Icterohaemorragica	RGA
3	L. canicola	Canicola	HV IV
4	L. hardjo	Hardjo	Hardjo prajinto
5	L. hebdomedis	Hebdomedis	Hebdomedis
6	L.grippotyphosa	Grippotyphosa	Moskova
7	L.javanica	Poi	Poi
8	L. patoc	Patoc	Patoc I

Table 2: Seroprevalence of Leptospiral antibodies in different Wild animals in Andhra Pradesh

S.No	Species	Total samples screened	No Positives	Percentage positivity	Serovars		
					janica	ictero	hardjo
1	Jackals	16	6	37.5	4 (66.6)	2 (33.3)	-
2	Hyenas	8	1	12.5	-	1 (100)	-
3	Deer	14	6	42.9	-	3 (50)	3 (50)
4	Lion	4	-	-	-	-	-
5	Elephants	6	2	33.3	-	-	2 (100)
TOTAL		48	15	31.25			