

Seroprevalence of leptospirosis among captive Asian elephants in Kerala, India - a short communication

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ABSTRACT

Leptospirosis is an infectious disease of zoonotic significance, caused by distinct members of the genus *Leptospira*, with a broad spectrum of hosts, including domestic and wild animals. The present paper reports the seroprevalence of leptospirosis among captive Asian elephants in Kerala. Whole blood samples were collected from fifty, apparently healthy captive elephants from different districts of Kerala state. The serum was separated from the whole blood and subjected to the Microscopic Agglutination Test (MAT) for detection of antibodies to different serovars of *Leptospira*. The test was carried out using actively grown cultures of twelve common leptospiral serovars. Out of the total of fifty serum samples screened by MAT, 19 samples (38 per cent) showed a positive reaction with antibody titres in a 1:200 dilution. The prevalent serovars were Pomona, Icterohaemorrhagiae, Hebdomadis, Grippotyphosa and Canicola, and the highest prevalence was noticed for the serovar Pomona. The positive results indicated the presence of infection or the previous exposure of captive elephants to leptospiral antigens. The high rate of prevalence of leptospiral antibodies in captive elephants warrants the monitoring of these animals for clinical disease and adopting the necessary control strategies for preventing this re-emerging zoonotic disease.

Key words: leptospirosis; seroprevalence; MAT; elephants

Introduction

Kerala is considered endemic for leptospirosis among humans and animals. Leptospirosis is one of the re-emerging zoonotic diseases, and the role

of rodents and domestic animals in the maintenance and transmission of the disease has been proved (PATIL et al., 2014). Leptospirosis has been

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reported in a wide variety of animals, including cattle, dogs, pigs, horses, sheep and goats. Reports of leptospirosis in wild animals in Kerala are scanty. The disease also has zoonotic significance, and human beings are considered to be end hosts. Rodents, especially rats, are the reservoir hosts for leptospirosis. Infection is acquired through contact with water and soil contaminated with the urine of infected animals, as well as via direct contact. Even though cultural isolation and identification is the best confirmatory test for diagnosis, it is laborious and time consuming. The microscopic agglutination test is a highly sensitive and specific test, which enables serovar-specific and quantitative diagnosis of leptospirosis, based on antibody detection. It is considered as the gold standard test (OIE, 2014).

There are more than 250 pathogenic serovars of *Leptospira interrogans*. Even though there are reports of the seroprevalence of leptospiral antibodies among humans (AMELDEV et al., 2019) and animal species in Kerala, such as cattle (TRESAMOL et al., 2018), goats (DHIVAHAR et al., 2019), dogs (AMBILY et al., 2013; VAMSHIKRISHNA et al., 2013) and pigs (RESHMA et al., 2018), there are no reports of seroprevalence among elephants in Kerala. There are also reports of the seroprevalence of leptospirosis among captive Asian elephants from other states of India (SHIVRAJ et al., 2009; KOTEESWARAN, 2006; RANI PRAMEELA et al., 2015). The incidence of the clinical disease in a captive Asian elephant in Tamil Nadu was reported by JAYATHANGARAJ et al. (2015). Recently, ATHAPATTU et al. (2019) demonstrated shedding of pathogenic leptospires in the urine of four out of 13 healthy elephants in Sri Lanka, and suggested the possibility of their role as a source of infection. Hence the present study was undertaken to assess the seroprevalence of leptospirosis among captive Asian elephants in Kerala.

Materials and methods

Whole blood samples were collected from fifty, apparently healthy, captive elephants from different districts of Kerala state. The serum was separated from the whole blood and subjected to the Microscopic Agglutination Test (MAT) for

detection of antibodies to different serovars of *Leptospira*. The test was carried out using actively grown cultures of twelve common leptospiral serovars. The serovars included in the antigen panel were: *Leptospira interrogans* serovars Australis, Autumnalis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Pyrogenes, Bataviae, Hebdomadis Tarassovi, Javanica and Sejroe. A 1:100 serum dilution was prepared in sterile PBS (Hi Media, India), 30 µL of which was placed in 96 well microtiter plates (Tarsons) and mixed separately with 30 µL of each of the five-to ten-day-old live *Leptospira* serovars. Antigen controls were set with 30 µL sterile PBS and 30 µL of different live *Leptospira* serovars, and the plates were incubated at 37 °C for two to four hours. After incubation, the results were read by examining a drop of the serum-antigen mixture from each well under a dark field microscope at low power, for agglutination of leptospires. The end point was recorded as the highest dilution of the serum showing 50 per cent agglutination or a reduction in number of organisms, in comparison to the respective antigen control. Further, quantitative assay was carried out in 96 well microtiter plates against the reacting serovars of leptospires. All the 96 wells were filled with 30 µL PBS. In the first well of each row, 30 µL of 1 in 50 dilution serum samples were added and mixed. Then, serial double fold dilutions were made up in eight wells and 30 µL was discarded from the last well. A constant volume of 30 µL of a particular serovar, with a density of 2×10^8 per mL, was added in each row and incubated at 37 °C for two to four hours. All the final dilution mixtures (100, 200, 400, 800, 1600, 3200, 6400, 12800) were observed under a dark field microscope and the results were recorded. The reciprocal of the highest dilution of the serum which showed 50 per cent agglutination or 50 per cent reduction in the number of free leptospires in comparison to the control, was considered as the respective titre.

Results and discussion

Out of the total of fifty serum samples screened by MAT, 19 samples (38 per cent) showed positive reactions with antibody titres in a 1:200 dilution.

Microscopic Agglutination Test is the gold standard test for the diagnosis of leptospirosis, and it is a useful herd test for identification of the prevalent serovars in an area. The serovars identified in the present study were: Pomona (47.37%), Icterohaemorrhagiae (21.05%), Grippotyphosa (5.26%), Hebdomadis (15.79%) and Canicola (10.53%) (Table 1). There are several reports of seroprevalence among captive Asian elephants from other states in India. VENGADABADY et al. (2009) reported the seroprevalence of leptospirosis among 41 out of 42 captive Asian elephants

in Anamalai and Mudhumalai forest ranges in Tamil Nadu, India, and the serovars included Australis, Hardjo, Hebdomadis, Javanica, Pomona, Pyrogenes and Tarassovi. KOTEESWARAN (2006) reported the seroprevalence of leptospirosis among wild animals in captivity, including elephants, and serovar Tarassovi was predominant (30.43%), followed by serovars Javanica (26.09%), Pyrogenes (17.39%), Australis (8.69%) and Sejroe, Hebdomadis, Icterohaemorrhagiae and Pomona (4.35% each).

Table 1. The number of samples positive for different serovars

Sl.No.	Serovars	Positive samples	Percentage
1	L.Pomona	9	47.37
2	L. icterohaemorrhagiae	4	21.05
3	L. Grippotyphosa	1	5.26
4	L. hebdomadis	3	15.79
5	L. Canicola	2	10.53
	Total	19	100

Among the 12 *Leptospira* reference serovars used in the study, the *Leptospira interrogans* serovar Pomona was identified as the most prevalent serovar, followed by Icterohaemorrhagiae, Hebdomadis, Canicola and Grippotyphosa.

SHIVRAJ et al. (2009) found serovars of *Leptospira interrogans*, Australis and Canicola by MAT in captive elephants in Karnataka, with an overall prevalence of 13.72 per cent among 51 animals kept in the three different forest ranges, that is, Bandipur, Shimogga and Mudhumalai. In northern Thailand, a higher seroprevalence of 58 per cent was reported with the prevalent serovars *Leptospira interrogans* serovars Sejroe, Tarassovi, and Ranarum, whereas in western Thailand, the seroprevalence was 57 per cent and the prevalent serovars were Tarassovi, Sejroe, Ranarum, Bataviae and Shermani (ONI et al., 2007). These results were similar to studies in domestic livestock and stray dogs in the Bangkok district. The presence of antibodies against *Leptospira Canicola* in elephant

sera was reported by NARAYANA BHATT et al., (1998) and they suggested the possible role of wild canines or other animals. ARORA (2003) reported the seroprevalence of *Leptospira* serovars Volbuzzi and Pyrogenes in apparently healthy captive elephants, and further mentioned the occurrence of different leptospiral serovars in elephants, such as: Pomona, Icterohaemorrhagiae, Grippotyphosa, Hebdomadis, Hardjo, Canicola etc.

Reports on the seroprevalence of leptospirosis in other domestic animals in Kerala have also revealed almost similar findings. The most predominant serovars among pigs in Kerala included Pomona (45.95 per cent), Grippotyphosa (24.32 per cent), Canicola (13.51 per cent), Icterohaemorrhagiae (10.81 per cent) and Tarassovi (5.41 per cent) (RESHMA et al., 2018). AMBILY et al., (2013) observed *Leptospira interrogans* serovar Autumnalis as the most prevalent serovar among dogs in Kerala, followed by Australis, Pomona, Canicola, Pyrogens, Icterohaemorrhagiae, Javanica

and Patoc. The study by SOMAN et al. (2014) identified the serovars Pomona, and Australis as common ones infecting humans, animals, and rodents, in central and North Kerala, by serology and isolation.

None of the elephants under study had any clinical signs of the disease. All were apparently healthy. FOWLER and MIKOTA (2006) stated that elephants could develop a positive titre to one or more sero groups of leptospires, but no clinical disease occurred. GOVINDARJAN et al., (2006) reported anorexia, icteric mucous membrane, and yellow coloured urine as the clinical signs in an infected elephant.

The results of the present study reveal a similar pattern of occurrence of various serovars as in other domestic animals, rodents and humans in Kerala. So the sources of infection will be infected animals and rodents. Water bodies for drinking or wallowing purposes for elephants might have been contaminated with the urine of infected animals or rodents, and serve as a source of exposure. The recent report by ATHAPATTU et al. (2019) demonstrated the shedding of pathogenic leptospires in the urine of four out of 13 healthy elephants in Sri Lanka, and suggested the possibility of their role as a source of infection. Domestic animals maintain the disease because of continued exposure to asymptomatic carriers or transmission within herds (GREVEMEYER et al., 2017). LILENBAUM et al. (2009) reported carrier status among goats and sheep with continuous or intermittent excretion of leptospires in their urine. Carriers play an important role in the transmission of leptospirosis between animals or humans.

The positive results indicated the presence of infection or the previous exposure of these captive elephants to leptospiral antigens. The high rate of prevalence of leptospiral antibodies in captive elephants warrants monitoring these animals for clinical disease and adopting the necessary control strategies for preventing this re-emerging zoonotic disease. It is also recommended to screen all domesticated elephants that are in close contact with humans, for the shedding of pathogenic leptospires.

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SAŽETAK

Leptospiroza je zarazna bolest od zoonotske važnosti uzrokovana različitim pripadnicima roda *Leptospira*, koja ima velik raspon domaćina, uključujući domaće i divlje životinje. U ovom je radu prikazana seroprevalencija leptospiroze među zatočenim azijskim slonovima u Kerala u Indiji. Uzorci pune krvi prikupljeni su od naizgled zdravih pedeset zatočenih slonova iz različitih okruga Kerale. Serum je odvojen od pune krvi i podvrgnut mikroskopskom testu aglutinacije (MAT) radi otkrivanja protutijela na različite serovarove leptospira. Test je proveden uz upotrebu aktivno uzgojenih 12 učestalijih serovarova leptospira. Od ukupno pedeset uzoraka seruma analiziranih MAT-om 19 uzoraka (38 %) pokazalo je pozitivnu reakciju s titrom protutijela pri razrjeđenju 1:200. Najučestaliji su serovarovi bili Pomona, Icerohaemorrhagiae, Hebdomadis, Grippotyphosa i Canicola, s najvećom uočenom prevalencijom za serovar Pomona. Pozitivni rezultati upućuju na prisutnost infekcije ili prethodnu izloženost zatočenih slonova antigenima leptospira. Veća stopa prevalencije na protutijela leptospira u zatočenih slonova upozorava na potrebu za praćenjem ovih životinja s obzirom na kliničku bolest te usvajanje potrebnih strategija kontrole radi prevencije ove reemergentne zoonoze.

Ključne riječi: leptospiroza; seroprevalencija; MAT; slonovi
