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ORIGINAL RESEARCH

Canine leptospirosis – a seroprevalence study from Kerala, India

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Abstract

Aim: To study the seroprevalence of leptospirosis in dogs in Kerala and to identify the most prevalent serovar.

Materials and Methods: A total of 205 sera collected from dogs were screened for the presence of antibodies against leptospirosis by Microscopic Agglutination Test (MAT).

Results: A seroprevalence rate of 71.12 per cent was observed. *Leptospira interrogans* serovar Autumnalis was found to be the most prevalent serovar followed by Australis, Pomona, Canicola, Pyrogenes, Icterohaemorrhagiae, Javanica and Patoc.

Conclusions: The results revealed the high prevalence of anti leptospiral antibodies in dogs in Kerala. The emergence of serovars other than the vaccinal serovars necessitates the incorporation of these in the vaccines because the immunity against leptospirosis is serovar specific.

Key words: canine leptospirosis, MAT, seroprevalence

Introduction

Leptospirosis, a spirochaetal zoonosis, has emerged as a serious global veterinary and public health problem. The disease has gained much importance as it is often undiagnosed [1]. Many places in South India, including Kerala, are known to be endemic for leptospirosis [2]. The pathogenic leptospires are divided into more than 250 different serovars and the most common serovars affecting dogs include Canicola, Icterohaemorrhagiae, Pomona, Bratislava, Grippotyphosa and Australis [3]. Despite routine vaccinations, there are increasing reports of canine leptospirosis in Kerala. The commercial vaccines are becoming less effective because the immunity is serovar specific and new serovars are emerging [4]. Therefore for successful immunization, it is essential to identify the most predominant serovar in the specific region. Hence, the present study was undertaken to identify the prevalence of various serovars involved in canine leptospirosis in three regions of Kerala *viz.*, Thrissur, Palakkad and Kozhikode.

Materials and Methods

A total of 205 canine serum samples were collected from three regions of Kerala *viz.*, Thrissur, Kozhikode and Palakkad during a period from March, 2011 to February, 2012. The samples included those collected from 35 healthy vaccinated animals and 30 healthy unvaccinated animals. The rest of the samples were taken from cases suspected for acute leptospirosis. Paired samples were collected from eight dogs which were diagnosed as positive for acute leptospirosis.

All the samples were subjected to Microscopic Agglutination Test (MAT). A battery of eight pathogenic serovars *viz.*, *Leptospira interrogans* serovar Australis, Autumnalis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Javanica, Pomona and Pyrogenes and non pathogenic Patoc I strain of L. *biflexa* serovar Patoc procured from the National *Leptospira* Reference centre, Regional Medical Research Centre, Port Blair, Andaman and Nicobar Islands were used as antigens in the test. These cultures were maintained in *Leptospira* liquid culture medium (Difco) with bovine serum albumin supplement. The test was carried out in a 96 well U-bottom microtitre plates. Test sera were first diluted to 1:50 and then serially diluted two fold in phosphate buffered saline, to obtain dilutions of 1: 100 to 1: 25600. To 30 µl each of the serum dilution, 30 µl of six day old live antigen was added. Appropriate antigen controls were set with 30 µl PBS and 30 µl of antigen and the plates were incubated at 37°C for two hours. After incubation, the result was read by examining a drop of serum-antigen mixture from each well under dark field microscope. The antibody titre was the highest dilution of serum showing agglutination of 50 per cent or more leptospiral organisms. Reciprocal agglutination titres of greater than or equal to 100 were considered as positive reactions [5]. The serovar reacting at the highest titre was considered to be the infecting serovar. Sera samples showing same agglutination titres to more than one serovar were considered as mixed equals.

Results

One hundred and forty six samples (71.12 per

cent) including the 30 healthy vaccinated animals were found to be positive by MAT. The sera from 35 healthy unvaccinated animals were found to be negative. In vaccinated healthy animals, agglutination was observed against serovars Canicola and Icterohaemorrhagiae which are included in the common commercial vaccine. Among the eight paired serum samples collected, except one all showed a four fold increase in MAT titre.

The MAT titres varied from 1: 100 to 1: 6400. The most predominant serovar was found to be Autumnalis followed by Australis, Pomona, Grippotyphosa, Canicola, Pyrogenes, Icterohaemorrhagiae, Javanica, and Patoc (Table-1). Five samples were found to be mixed equals ie. equal titre was noticed one each for Icterohaemorrhagiae and Autumnalis, Grippotyphosa and Icterohaemorrhagiae, Canicola and Icterohaemorrhagiae, Javanica and Pyrogenes and Canicola, Icterohaemorrhagiae and Pomona. The area wise prevalence of different serovars was not studied.

Table-1. Seroprevalence of canine leptospirosis

Sr. No.	Serovar	Positive	Per cent positive
1.	Autumnalis	36	23.97
2.	Australis	28	19.17
3.	Pomona	20	13.69
4.	Grippotyphosa	14	8.9
5.	Canicola	14	8.9
6.	Pyrogenes	12	7.5
7.	Icterohaemorrhagiae	13	8.2
8.	Javanica	9	6.1
9.	Patoc	5	3.4
	Total	146	

Discussion

Microscopic Agglutination test is considered as the gold standard test for the diagnosis of leptospirosis. The test is serovar-specific and provides useful epidemiological data in the form of presumptive serovars. A titre of 1:100 and above is considered as positive in MAT [5]. In the present study, 146 (71.12 per cent) sera had titre above this. However, in an endemic region, where vaccination is routinely practiced, this titre has to be increased to confirm the presence of acute infection [6] as there were antibodies in healthy vaccinated animals.

The study reveals the emergence of Autumnalis as the most predominant serovar in the region. Similar results were obtained in a survey of canine leptospirosis on the island of Barbados, where Autumnalis was found to be the most common reactor, followed by serogroups Icterohaemorrhagiae and Australis and Pomona [7]. The epidemiological studies on leptospirosis in different parts of the world have proved that majority of human disease was caused by *L. interrogans* serovar Autumnalis [8], [9]. Dogs are considered as maintenance hosts for serovar Canicola and incidental hosts for other serovars and are a potential source of infection for human beings in contact with them. In fact, they are acting as a link between the reservoirs of infection in the environment and the human beings.

According to various studies conducted in southern states of India, *Australis* was reported to be the emerging serovar [3] [10]. A seroprevalence study conducted in Kerala in 2004 revealed Pomona as the most prevalent serovar affecting dogs [11]. However, in the recent studies, serovars Australis and Autumnalis were found to be more predominant here [12], [13], [14]. Variation could be noticed over years in the prevalence of leptospirosis in animals. Most of the above studies in Kerala were restricted to Thrissur district only. Hence, a detailed seroprevalence study is necessary covering the entire state. The emergence of a serovars other than the vaccinal serovars necessitates the incorporation of these in the vaccines because the immunity against leptospirosis is mostly serovar specific.

Conclusions

Though the current vaccines against leptospirosis in Kerala contains the serovars Icterohaemorrhagiae and Canicola, the present study reveals the need of incorporating the serovar Autumnalis as it was found to be greatly associated with the disease in Kerala. The high seroprevalence of canine leptospirosis is of public health concern because close contact with dogs may connect susceptible individuals to environmental reservoirs of infection.

Authors' contribution

RA carried out the study and prepared the article. MM and SJ participated in scientific discussion, SVK and GA assisted in conducting MAT. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interests.

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