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Research Article

Seroprevalence of *Leptospira* Spp. in Clinically Healthy Horses in Chennai, India

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Abstract

Background and Objective: The number of Leptospirosis outbreak in horses was increasing in recent years in Chennai, India. There are reports in other parts of the world about the prevalence of this organism in clinically healthy horses. Hence, the present study was undertaken to assess the seroprevalence of *Leptospira* spp. in clinically healthy horses and to find out the association among age, gender and serovar type in Chennai, India. **Methodology:** Sera samples collected from horses (n=162) in and around Chennai were tested for a battery (n=12) of *Leptospira* antigens using Microscopic Agglutination Test (MAT). **Results:** Out of 162 (Male:40, Female:122) samples tested, 126 (77.77%) were positive out of which 19.84% were males and 80.16% were female horses, respectively. Among the 40 male horses 25 (62.50%) were positive and among the 122 females 101 (82.79%) horses were positive. **Conclusion:** The most of the healthy horses in Chennai, India are seropositive carriers for asymptomatic Leptospiral infection. It should be viewed seriously under zoonoses perspective.

Key words: *Leptospira* spp., seroprevalence, zoonosis, healthy horse, serovars, asymptomatic

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Leptospirosis is a global veterinary and public health problem. It is caused by infection with one of more than 12 pathogenic species of *Leptospira*, although *L. australis*, *L. autumnalis* and *L. interrogans* are responsible for the majority of infections¹. The prevalence of Leptospiral infection was 76.05% in horses and the titer levels between 100 and 200 were positive for *Leptospira* was reported earlier². Infection usually results from direct transmission via contaminated urine or placental fluid or indirectly from a contaminated environment. The biodiversity of Leptospirae in the environment is affected by geography, climate, biotic interactions and anthropogenic activities³. The disease occurs in domestic animals and humans inducing fever, hemoglobinuria, jaundice, abortion, mastitis, reduced milk production, reproductive disorders and death⁴. The number of Leptospirosis outbreak in horses were increasing in recent years in Chennai, India. There are reports in other parts of the world about the prevalence of this organism in clinically healthy horses. Horses present a limited number of diseases that can be transmitted to humans, however, the bond established between horses and their owners allows a closer contact with them than with other large animals. This closer bond can increase the exposure rate to *Leptospira* spp., for example, Leptospirosis is a worldwide zoonosis caused by the infection of *Leptospira interrogans* serovars⁵. The most common serogroup detected was *Laptospira pomona* (*L. pomona*) followed by *Laptospira grippotyphosa* (*L. grippotyphosa*). Uveitis is the most frequently encountered clinical manifestation of Leptospirosis in horses, however, abortion and stillbirth are serious problems. The pathogenic serovars can be transmitted through infected urine, contaminated soil or water and other bodily fluids¹, affecting humans, domestic animals and wildlife. Although Leptospirosis is a systemic disease more common in humans and domestic animals, mainly dogs, cattle and swine⁶, it can also occur in horses. Little attention is commonly given to the potential role horses play in zoonotic diseases¹. Though no horses presented clinical signs of Leptospirosis, the study confirms exposure to *Leptospira* spp. and the importance of studying in more detail the livelihood conditions in which the horses are kept and possible risk of transmission to their owners and other horses. Hence, the present study was undertaken to assess the seroprevalence of *Leptospira* spp. in clinically healthy horses and to find out the association among age, gender and serovar type in Chennai, India.

MATERIALS AND METHODS

Blood samples were collected from 162 horses (122 females and 40 males) maintained at a private farm (114), Officers Training Academy (25) and Greater Chennai Police - Mounted Branch (23) during the period June-July, 2013. On the basis of age, these horses were divided into four groups, viz., Group I: 3-6 years, Group II: 7-10 years, Group III: 11-14 years and Group IV: 15-18 years. None of these animals had been vaccinated against Leptospirosis and there was no history of Leptospirosis related symptoms or signs of illness at the time of sampling. About 5 mL of blood was collected from jugular vein by using a vacutainer and was allowed to clot. Further, it was centrifuged at 3000 rpm for 10 min, serum was separated and stored at -18°C until tested.

The sera samples were tested by Microscopic Agglutination Test (MAT) for a battery of 12 *Leptospira* antigens, including, Australis, Autumnalis, Icterohaemorrhagiae, Canicola, Grippotyphosa, Habdomadis, Pyrogenes, Pomona, Tarassovi, Ballum, Javanica and Hardjo. The MAT titre of >1:100 was considered as seropositive. The MAT was done at Zoonoses Research Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, 600051 Chennai.

Statistical analysis: The results were analyzed by chi-square test to determine whether sex and age of the horses was significantly related to the prevalence of Leptospiral antibodies.

RESULTS

Out of the 162 sera samples collected from apparently healthy horses, 126 (77.77%) had agglutinating antibodies against one or more serovars (sv) of *Leptospira* and were considered seropositive, out of which 19.84% were males and 80.16% were female horses, respectively. Among the 40 male horses 25 (62.50%) were positive and among the 122 females 101 (82.79%) horses were positive. The prevalence of other serovars and distribution of MAT titres among the samples that tested positive are presented in Table 1. Titres ranged from 1:100 to 1:1600. Some animals were reactive to more than one serovar viz., 28 horses reacted for 2 serovar, 27 horses for 3 serovar, 17 horses for 4 serovar, 5 horses for 5 serovar and one horse for 6 serovar. Twenty five male horses (62.50%) and 101 females (82.79%) were positive by MAT.

Table 1: Prevalence of other serovars and distribution of MAT titres among the samples that tested positive

Serovar	MAT titre and number of horses					Total	Percent
	100	200	400	800	1600		
Australis	38	15	7	12	1	73	57.94
Autumnalis	29	8	3	1		41	32.54
Javanica	29	5	2			36	28.57
Tarassovi	21	10				31	24.60
Pyrogenes	21	1	2	1		25	19.84
Icterohaemorrhagiae	10	2	1			13	10.32
Pomona	7	4	2			13	10.32
Canicola	2	1	2	1		6	4.76
Habdomadis	5					5	3.97
Ballum	5					5	3.97
Grippotyphosa	1					1	0.79
Harjo						0	0.00
Total	168	46	19	15	1	249*	

*Some samples were positive for more than one antigen

DISCUSSION

The objective of this study was to determine the prevalence of *Leptospira* serovars among the horses population in and around Chennai. Earlier, Rao *et al.*⁷ studied the seroprevalence of Leptospirosis in horses (n = 466) in Hyderabad, India and Koteleswaran⁸ studied in 4 horses in Chennai. Seropositivity rates and serovars present in horses vary among studies. The prevalence of *Leptospira* serovars in this study was 77.77%, which is comparable with the other reports worldwide, that reported⁹ prevalences ranged from 1.5-79%. Though Australis and Autumnalis serovars were in high prevalence in India, others reported that the serovars vary between Icterohaemorrhagiae, Bratislava, Copenhageni, Sejroe, Australis and Pomona⁹. Differences in seroprevalence may be related to the number of horses tested, the detection of nonpathogenic serovars and the inclusion of titers $\geq 1:20$ as positive samples. In the present study⁹ a dilution of $>1:100$ was used as a cutoff point for seropositivity. Most researchers used the MAT titre as a diagnostic tool for serology in equine Leptospirosis and considered a dilution of 1:100 as positive^{10,11}.

Wide range of prevalence of sv Bratislava to the extent of 40.70% was recorded in New York⁹, 44.75% in Croatia⁹ and Jung *et al.*¹² in Korea, sv Grippotyphosa, 33.33% in Tabriz^{10,13}, Iran, sv Hardjo, 43% in Ardabil¹⁴, Iran and sv Pomona (40/466) in Hyderabad⁷, India. In the present study it was found sv Australis to be the most prevalent serovar of *Leptospira* in Chennai, India unlike the previous studies reported.

The second most prevalent serovar recorded in this study was sv Autumnalis (32.54%) followed by sv Javanica (28.57%)

and sv Tarassovi (24.60%). These findings were also in contrast to findings of Turk *et al.*¹⁵, where they recorded sv Pomona (18.20%) as the second most serovar in a study of 8,059 horses in Croatia.

However, it is important to keep it in mind that the prevalence of serovars could vary among region, due to climate and geographical variations⁶. Some studies specifically performed in horses showed a seroprevalence that varied from 1-95%, depending on the geographic location and the serovars assessed, indicating that exposure was significantly more common than the clinical disease. This underlines the low level of systemic inflammatory response in horses with subclinical Leptospirosis¹¹.

However, there is a permanent risk of convalescent carriers excreting Leptospire from their kidney, especially with a large volume of daily urine content. Hence, they have a high potential for contamination of the environment¹⁵. In present study, it was found that serological studies for Leptospirosis such as MAT, often indicated more than one serovar. This might be the result of mixed serovar infection but the existence of cross reactivity in the MAT between the serovars was well known^{14,16} and can be excluded from this interpretation.

In seropositive animals, there was a significant ($p < 0.01$) difference between males and females which was in agreement with others^{13,17}. However these findings were in contrast to Jung *et al.*¹², who observed no gender related differences. In this study there was no significant relationship among age groups and the prevalence of Leptospiral infections, which was in accordance with the findings of others^{10,12,17}. Little attention is commonly given to the potential role horses play in zoonotic diseases. This study

provides evidence that clinically healthy horse with *Leptospira* spp. is common and that serovars causing clinical disease in humans.

CONCLUSION

The present study confirmed that existence of Leptospiral infection in horse populations in Chennai, India. In addition, these results confirmed that the majority of the Leptospiral infection in horses was asymptomatic and the animals could act as carriers. Presence of antibodies in the absence of infection indicated exposure to the organism. This may not only help to prevent equine infection but infection of other mammals as well, including humans.

SIGNIFICANCE STATEMENT

This study discovers the possible seroprevalence of *Leptospira* spp. organism in horses which is clinically healthy. However there are possibilities that these organisms may flare up and end up in clinical infection during stress conditions. Hence, this study will help the stud farm owners and equine veterinarians to understand the importance of seroprevalence and help them to take appropriate preventive measures in future.

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