



Short Communication

Seroprevalence of *Borrelia burgdorferi sensu lato* in Camel (*Camelus dromedarius*) in Punjab, Pakistan

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ABSTRACT

A seroprevalence study was conducted on the presence of *Borrelia burgdorferi sensu lato* antibodies in 405 camels of two districts of Punjab, Pakistan i.e., Bhakkar and Bahawalpur from May 2019 to January 2021. A questionnaire was used to collect data regarding potential risk factors like gender, age and tick infestation. Serological examination revealed the positive percentage of *B. burgdorferi sensu lato* in camels was 2.47% (10/405). Risk factor analysis showed that gender, age and tick infestation are significantly ($p < 0.05$) associated with occurrence of borreliosis in camels. This study may play an important role in the transmission of borreliosis in understanding of other animal species as well as humans in Pakistan.

Article Information

Received 11 July 2021

Revised 05 August 2021

Accepted 21 August 2021

Available online 12 November 2021
(early access)

Published 26 April 2022

Authors' Contribution

NR conducted the research. AZD supervised the research. MHS and AAS were members of the supervisory committee. MU statistically analysed the data, drafted the manuscript and proofread it. QM helped in laboratory work. MZI and MR helped in collection of samples from the field.

Key words

Camel, *Borrelia burgdorferi sensu lato*, ELISA, Risk factors

The camelid family, *Camelus* genus comprises three species: *Camelus bactrianus*, *Camelus bactrianus ferus* and *Camelus dromedaries* (Liu *et al.*, 2015). Camels play a significant role in milk and meat production. (Pasha *et al.*, 2013). Camel population is around 18.58 million all over the world, while in Pakistan 1.2 million camels are reared in desert and semi desert area (Ministry of Finance, Pakistan Economic Survey, 2018-19). Various camel diseases including Middle East respiratory syndrome coronavirus, borreliosis, trypanosomiasis, theileriosis and babesiosis pose a significant threat to public health. Numerous studies have shown that dromedary camel is an intermediate host and the major cause of zoonotic diseases (Mohammadpour *et al.*, 2020).

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0030-9923/2022/0004-1987 \$ 9.00/0



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Borreliosis is one the most significant zoonotic diseases endemic and geographically distributed in central Asia, United States and Eastern Europe (WHO, 2020). This disease has been neglected in camel population of Pakistan although it has reported in camels (2.6%) neighboring China. The spirochaete *Borrelia burgdorferi sensu lato* having helical-shape has been reported to be causative agent of this disease. *B. burgdorferi sensu lato* consists of 21 genospecies (Kingry *et al.*, 2018), whereas mainly three, *B. afzelii*, *B. garinii* and *B. burgdorferi sensu stricto* are of public health importance (Barbour *et al.*, 2005). Ticks are significant vectors for *B. burgdorferi sensu lato* in domestic and wild animals (Elhelw *et al.*, 2021). *Ixodes ricinus* in Europe and *Ixodes scapularis* and *pacificus* species are the major species of hard ticks in the northwestern and eastern in the United States, respectively (Labrini *et al.*, 2021). Clinical signs of borreliosis in camels include fever, erythema migrans, weight loss, encephalitis, including sporadic lameness and arthritis (Maraspin *et al.*, 2021).

Diagnosis of borreliosis was performed through various techniques such as Giemsa staining, ELISA and PCR. ELISA is the best method for diagnosis of borreliosis in short time for its accuracy. The aim of current study was

to detect presence of *B. burgdorferi sensu lato* antibodies in camels of two districts of Punjab along with analysis of risk factors. This study will help in the diagnosis of zoonotic vector borne disease of borreliosis in camels.

Materials and methods

The present study was carried out in two sites of arid zone (Cholistan and Thal) of the two *viz.* Bhakkar (31.6082° N, 71.0854° E) and Bahawalpur (29.3544° N, 71.6911° E) of Punjab province, Pakistan from May 2019 to January 2021.

Blood samples (1ml) collected from four hundred and five dromedary camels in vacutainer (Franklin Lakes, USA) were centrifuged at 1300–1800rpm for 20 min for separation of serum.

The study were conducted after approval of the ethical committee, University of Veterinary and Animal Sciences, vide letter NO. 894, dated 22-08-2017.

Questionnaire was used to collect data on possible risk factors containing gender, infestation and age of camels in study area.

Enzyme-linked immunosorbent assay kit (SNAP® 4Dx® Plus Test Kit, IDEXX Laboratories, USA) was used for diagnosis of *B. burgdorferi sensu lato* the manufacturer's instructions. Sera of camels were analyzed by the ELISA for IgM and IgG antibodies using a commercial set "Enzygnost Borreliosis" (Behring, Marburg, Germany). In this protocol, the antigen mixture contains 100 kD, 39 kD, 17 kD, Osp A, Osp B, 41 kD, and Osp C. This assay is highly sensitive. In this method ultrasonificate of *Treponema phagediens* was added in sample buffer which minimize the frequency of cross reactions. ELISA reader for this assay was used with a 450 nm filter.

The qualitative data collected were analyzed by Chi Square test. Odd ratio was calculated for determining association of potential risk factor. Statistical analysis was conducted on IBM SPSS software (version 20.0.0).

Results

Table I shows area wise prevalence of borreliosis in camels.

Out of 270 serum samples collected from district Bhakkar, 1.85% (5/270) samples were positive for *B. burgdorferi sensu lato* while out of 135 serum samples, 3.70% (5/135) were positive for *B. burgdorferi sensu lato*.

Table I shows that collected from Bhakkar the highest positive cases was seen from tehsil Mankera (2.5%) and Kaloorkot (3.33%). The blood sera from tehsils, Darya Khan and Bhakkar were 100% negative for *Borrelia burgdorferi sensu lato* in camel. Out of 135 serum samples collected from Bahawalpur, the highest positive samples were seen in tehsil Yazman (11.11%). Tehsil Hasilpur and Khairpur Tame Wali showed only one positive case

for *Borrelia burgdorferi sensu lato*, respectively. Tehsil Bahawalpur, Ahmed Pur Sharqia were 100% negative for borrelia.

Table II risk factors associated with *B. burgdorferi sensu lato* in camels.

Table I. Area wise prevalence of borreliosis in camels.

District	Tehsil (prevalence %)	Total	Positive (%)
Bhakkar	Mankera	120	3(2.5%)
	Darya khan	60	0(0%)
	Kaloorkot	60	2(3.33%)
	Bhakkar	30	0(0%)
	Subtotal	270	5 (1.85%)
Bahawalpur	Yazman	24(88.89%)	3(11.11%)
	Khairpur Tamewali	27	1(3.70%)
	Ahmedpur Sharqia	27	0(0%)
	Hasilpur	27	1(3.70%)
	Bahawalpur	27	0(0%)
	Subtotal	135	5(3.7%)
Total		405	10

Table II. Risk factors analysis in field study against *Borrelia burgdorferi sensu lato* in camels.

Risk factors	Total	Positive (%)	OR	p-value	
Gender	Male	170	2(1.2%)	5.151	0.02
	Female	235	13(5.5%)		
Age	<1 years	57	2(3.51%)	2.082	0.0177
	(1-8) years	233	4(1.71%)	1	
	> 8 years	115	9(7.83%)	4.861	
Infestation	Infested	405	303	5.125	<0.0001

Statistical analysis showed that there was significant association found in gender, age and in tick infestation. However, the seroprevalence percentage of antibodies against *B. burgdorferi* was four time higher in > 8 years (odd ratio 4.861, p-value= 0.0177) of camels and below <1 years (odd ratio 2.082) of camels was two time less positive as compared to above eight years old camels. Gender wise risk factor analyzed that seroprevalence in female camels was 13(5.5%) with an odd ratio 5.15 compared with male camels was 1.2 % positive in serum samples.

Discussion

In this study, *B. burgdorferi sensu lato* was identified

in serum samples using ELISA. Out of 270 serum samples from Bhakkar, 1.85% were positive of *B. burgdorferi sensu lato* while out of 135 samples from Bahawalpur, 3.70% were positive of *B. burgdorferi sensu lato* in camel. Obaidat *et al.* (2020) have reported that 1.2% camels were positive for antibodies against *B. burgdorferi sensu lato*. Current study findings also agreed with the findings of Stoebel *et al.* (2003) who evaluated different species of camel (Alpaca, Llama and Two-humped camel species) for antibodies against *B. burgdorferi sensu lato* and found 4.54% positive in each species. A similar study conducted by Praharaj *et al.* (2008) reported 13% positive cases for *Borrelia burgdorferi* IgG antibody. Similar findings were reported by Samir *et al.* (2015) and Stoebel *et al.* (2003) who reported 20% and 10.4% samples reactive for *B. burgdorferi sensu lato*. Likiwise this positivity was reported to be 18.4% in Slovakia (Travnicek *et al.*, 2002), 47.8% in Egypt (Helmy, 2000) and 26.3% in China (Yang *et al.*, 2015).

In this study, 1.85% camels were found antibodies against *B. burgdorferi sensu lato* positive in district Bhakkar, while 3.70% (5/135) camels were positive for *B. burgdorferi sensu lato* antibodies in district Bahawalpur. Difference in positive percentage of borreliosis in different regions was also found worldwide including Canada (Gasmi *et al.*, 2017), USA (Schwartz *et al.*, 2017), England (Tulloch *et al.*, 2019), Germany (Dehnert *et al.*, 2012), Belgium (Geebelen *et al.*, 2019) and in Finland (Beek *et al.*, 2018). This difference may be due to climatic factors such as higher vegetation and humidity level that help in the life cycle of biological vector.

During gender wise study, female camels in this study were found, 5.5% positive for *B. burgdorferi sensu lato* while male camels, were 1.2% positive for *B. burgdorferi sensu lato*. Similar findings reported by Praharaj *et al.* (2008) showed higher prevalence in female camels as compared to male camels (15.86% Vs 10.95%). Seroprevalence between female and male was also observed in Belgium (Geebelen *et al.*, 2019), England (Tulloch *et al.*, 2019) and in Finland (Beek *et al.*, 2018). Another study in Sweden also revealed that females were at higher risk of infection than males (Bennet *et al.*, 2007). This higher percentage in females may be due to low number of male camels as our field study confirmed (Table I). Actually, people rear only one or two male camels, as compared to females, for the purpose of reproduction. Furthermore, the rate of tick infestation in male camels was also found lower than in female camels (Table I).

During field study, it was found that out of 405 camel, 74.81% were infested with ticks. Presence of ticks on camels was higher in Bahawalpur as compared to Bhakkar due to a number of factors including higher

camel population, less availability of feed and resultant compromised immune system. As ticks are the main vectors of *B. burgdorferi sensu lato* so, positive percentage of *B. burgdorferi sensu lato* was found significantly ($p < 0.0001$) higher in tick infested camels (Table I). Similar findings have been reported by Said *et al.* (2016) that 98% camels were infested with ticks and also reported by Barghash *et al.* (2016) that 53.78% camels were positive for tick infestation.

During age wise study, below one-year-old camel, 3.51% were positive for *B. burgdorferi sensu lato* while one to eight years old camel, 1.72% were positive for *B. burgdorferi sensu lato* and above eight years old camel, 7.83% (9/115) were positive for *B. burgdorferi sensu lato*. These findings supported Abdalla (2007) who reported that animals below one-year and above eight years were highly infected with *B. burgdorferi sensu lato*. An age wise seroprevalence was also reported in Canada (Gasmi *et al.*, 2017), England (Tulloch *et al.*, 2019), Germany (Wilking and Stark, 2009), and Belgium with highest prevalence in young and old age animals (Geebelen *et al.*, 2019). The positive percentage of *B. burgdorferi sensu lato* was found significantly ($p < 0.0177$) higher in animals of one year and above eight years of age.

Conclusion

In this study, we identified antibodies against *Borrelia burgdorferi sensu lato* in camel. Seroprevalence of *B. burgdorferi sensu lato* was found to be 2.47%. Different risk factors such as gender, infestation and age were found to be significantly associated with *B. burgdorferi sensu lato*. This pathogen may be part of the natural cycle in arid and Cholistan areas in Pakistan.

Acknowledgments

This study was part of Higher Education Commission approved project No. 1122 titled "Capacity building of vector-borne neglected diseases of livestock.

Data availability

The data will be made available on acceptable request to the corresponding author

Statement of conflict of interest

The authors have declared no conflict of interest.

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