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Short Communication

Occurrence of *Borrelia burgdorferi* Sensu Lato in Ticks on Camels, along with Risk Factors Analysis in Punjab, Pakistan

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ABSTRACT

Lyme borreliosis is multisystemic disease caused by *Borrelia burgdorferi* spirochete, which is tick-borne zoonotic disease of animals and human. It has worldwide circulation including Pakistan. The current study was designed to detect, the *B. burgdorferi* sensu lato in ticks on camels. Two hundred ticks collected (Bhakkar=100, Bahawalpur=100) from camel in Punjab were examined for potential risk factors, morphological identification, and molecular characterization by using polymerase chain reaction targeting 16S rRNA gene and phylogenetic analysis. *Hyalomma dromedarii* and *Rhipicephalus* were found to be 76% (152/200) and 24% (48/200), respectively. Molecular study showed the 10.5% (21/200) prevalence of *B. burgdorferi* sensu lato in ticks. Phylogeny showed that our isolates branched with isolates from tick (USA) and camel blood (China) with >80% bootstrap consensus. Risk factors examination showed that season, tick infestation and gender are highly significantly ($p < 0.05$) connected with the presence of *B. burgdorferi* sensu lato in ticks from camels during field study.

The dromedary camels are commonly prevalent in desert and semi desert area in Asia and North Africa (Ali *et al*., 2019). Population of camel all over the world is about 30 million (Zhu *et al*., 2019), and about 1.2 million camels in Pakistan (Pasha *et al*., 2013). Lyme borreliosis is recognize as a significant emerging tick-borne disease and is considered as plague of the 21st century. Lyme borreliosis is caused by various species of the *B. burgdorferi* sensu lato complex including *Borrelia afzelii, B. bavariensis, B. garinii* and *B. spielmanii* in Europe while it is caused by *Borrelia burgdorferi* sensu stricto in America (Perveen *et al*., 2021). This spirochete is transferred from ticks to camel, horse, human, and dog (Bhide et al., 2004; Torina et al., 2020).

Pakistan, being a semi-arid country, has a variety of different ticks. In camels, many hard ticks belonging to genera *Ixodes* have been reported including *Rhipicephalus*, *Argas*, *Amblyomma*, *Hyalomma*, *Ornithodoros* and *Dermacentor*. *B. burgdorferi* has been reported in ticks in many neighboring states including China, India, Iran and in United Arab Emirates (Tigani-Asil *et al*., 2021; Zhai *et al*., 2018; Kshirsagar and Ingale, 2014). Abundance of ticks, arid climate, maximum movement of camel and other animals between neighboring countries make the area vulnerable to transboundary tick-borne diseases like Lyme borreliosis. These diseases cause huge production and economic losses by compromising health of affected camels. Lyme borreliosis is one such tick-borne disease, which is neglected in Pakistan.

There is a lack of data on presence of *B. burgdorferi* sensu lato from ticks in Pakistan. Camel and ticks have a potential to transmit this pathogen to human population as well as other animals in Pakistan. In this background,

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the current study was planned to detect the *B. burgdorferi* sensu lato in ticks on camels in Punjab, Pakistan.

Materials and methods

The current study was conducted in two districts (Bhakkar and Bahawalpur) of Punjab, Pakistan. District Bhakkar, district has four tehsils, Darya khan, Kaloorkot, Mankera and Bhakkar. Two hundred ticks were collected, 100 from Bhakkar and 100 from Bahawalpur) from camel by using forceps and stored in absolute ethanol. Ticks were identified by standard key (Taylor *et al*., 2013; Alanazi et el., 2020) The study was conducted as per guidelines of Ethical Committee, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan (wide letter NO. 894, dated, 22-08-2017).

Ticks were homogenized in phosphate buffered solution (200µl) with micro pestle. Then homogenate was passed through a needle (27 guage) attached to 1ml syringe. Then DNA was extracted by using commercial QIAamp DNeasy Blood and tissue kit (QIAGEN, Maryland, and USA) from each homogenate.

The conserved 16s RNA gene was amplified using the following primers Forward 5′ AATAGGTTCTAATAATAGCCTTAATAGC 3′, and Reverse 5′ CTAGTGTTTTGCCATCTTCTTTGAAA 3′ (Zhai *et al*., 2018). Amplirun *Borrelia burgdorferi* DNA® control for optimization of assay was used (catalogues number MB076). The 30 µl PCR reaction mixture comprised forward Primer 3 µl, reverse primers 3 µl, 2X Master Mix (Thermo Scientific®) 15 µl, DNA sample 1.5 µl and then 7.5 µl water. The thermal cycle: Initial temperature 95 °C for 3 min followed by 32 cycles, each of denaturation at 95 °C for 3 seconds, annealing at 56 °C for 30 min, extension at 72 °C for 1 min, final extension at 72 °C for 15 min.

The PCR product was sequenced by Applied Biological Sciences. The DNA sequencings were compared with *B. burgdorferi* reference sequences through NCBI BLAST. BioEdit software were using for sequences alignment. In alignment, if sequences were similar then one of these sequences for phylogeny was processed. The phylogenetic tree was constructed with the already *B. burgdorferi* strains reported on GenBank NCBI by using MEGA6 software.

The qualitative data related to risk factors generated in the study was assessed using Chi Square test and odds ratio was calculated with IBM SPSS software version 20.

Results and discussion

The overall positive percentage of this pathogen was 10.5% in tick samples (21/200). In Bhakkar 7% (7/100) ticks while in Bahawalpur 14% (14/100) were found positive for *B. burgdorferi*. Many other scientists investigated presence of *B. burgdorferi* in *Hyalomma*, *Rhipicephalus* and *Ixodes* on camels (Adham *et al*., 2010; Elhelw *et al*., 2014; Alanazi *et al*., 2020; Perveen *et al*., 2021) and reported its prevalence from 1.8% to as high as 24% (Sarih *et al*., 2004; Said *et al*., 2016; Blazejak *et al*., 2018; Tigani-Asil *et al*., 2021; Michalski *et al*., 2021). The reason for this varied prevalence lies in the area of sampling. Grochowska *et al*. (2020) showed that infection with *B. burgdorferi* has higher rate of incidence in subtropical zones in hard ticks. Since Pakistan also occurs in subtropical region the prevalence of *B. burgdorferi* was found higher in ticks on camels.

Table I shows the potential risk factors analyzed during field study for the detection of *B. burgdoferi* sensu lato in ticks on camels. Adult ticks were 4.89 time more positive for *B. burgdorferi* sensu lato than nymphal stage of ticks. Out of thirty-five nymphs, only one nymph (2.85%) was found positive for *B. burgdorferi* sensu lato from camel while out of one hundred and sixty-five adult ticks, 12.12% (20/165) were found positive. This finding of current study coincides with that of Fatma *et al*. (2010). Positivity ratio in nymphs was low due to less sucking of blood from host at this stage. Adult ticks showed high detection rate for *B. burgdorferi* sensu lato because they suck more blood than nymph. Out of 270 camels from Bhakkar, 66.67% (180/270) camels while 135 camels from Bahawalpur, 91.11% (123/135) camels were infested. Out of 151 female ticks, 13.25% (20/151) ticks were positive for *B. burgdorferi* sensu lato from camels while out of 49 male ticks, only one tick was positive. This outcome of our study is in line with Kshirsagar and Ingale (2014).

Table I. Risk factors analysis during field study in ticks.

Risk factors	Total	Positive	OD	P value
Male	49	1(2.04%)	----	0.03
Female	151	20(13.25%	7.328	
Hyalomma	146	19(13.01%)	3.89	0.07
Rhipicephalus	54	2(3.70%)	----	
Nymph	35	1(2.85%)	----	0.0776
Adult	165	20(12.12%)	4.89	
Summer	77	3(3.90%)	----	0.04
Spring	43	5(11.635)	2.959	--
Fall	80	13(16.25%)	3.541	
Infestation		infested		<0.0001
Bhakkar	270	180(66.67%)	----	
Bahawalpur	135	123(91.11%)	5.125	

Out of 146 *Hyalomma*, 13.01% (19/146) were positive for *B. burgdorferi* sensu lato from camels while out of 54 *Rhipicephalus*, only two ticks were positive for *B. burgdorferi* sensu lato. Presence of *B. burgdorferi* sensu lato was significantly ($p < 0.04$) associated among different season. 3.90% (3/77) ticks were found positive for *B. burgdorferi* sensu lato in summer, 11.635% (5/43) in spring, while 16.25% (13/80) in fall season. These findings of the current study agreed by Roome *et al*. (2018) who found that, higher prevalence of *B. burgdorferi* in ticks in spring than in summer.

These isolates already described sequences on the NCBI-GenBank database. The data on IDs showed that accession number, country of origin and source of sample. Our sequences alignment based on their representation from ticks on camel. The isolated sequences from the ticks were 100% similar. Therefore, one sequence was included to measure the phylogeny of *B. burgdorferi* isolated from tick on camel in study area. Phylogeny showed that our isolates branched with isolates from tick (accession number. JF911486.1) and camel blood (accession numbers KY284020.1 and KY284015.1) with >80% bootstrap consensus. The analysis used nineteen sequences, including our sequence. A sequence of *B. turicatae* (accession number AY934610.1) was used as an outer group. In phylogenetic analysis, our sequences showed 100 percent similarity which may be due to circulation of a single strain of *Borrelia* in the study area and no mutation. So, there is a chance that this spirochete transferred from China to Iran from where it transferred to Pakistan. Another probability is that this pathogen transferred from India as it is a neighboring country.

Several methods are used for identifying the *B. burgdorferi* sensu lato complex. ELISA and culturing techniques cannot detect this pathogen in ticks because serum or body fluids are required for its detection in these methods. For accurate detection of *B. burgdorferi*. Polymerase chain reaction is used (Sazmand *et al*., 2019).

Conclusion

This is the first documented report of *B. burgdorferi* sensu lato spirochaetes in ticks on camels from Pakistan. *B. burgdorferi* sensu lato was detected in 10.5% (21/200) ticks collected from camels. *Hyalomma* and *Rhipicephalus* were found as greatest incriminated species of hard ticks in the transmission of spirochete *B. burgdorferi* sensu lato in Punjab, Pakistan. Risk factor analyses showed that gender, tick infestation and season were significantly ($p < 0.05$) associated with *B. burgdorferi* sensu lato in ticks. Further studies are required to observe the role of hard ticks and camels in the transmission of this pathogen to definitive hosts in Pakistan, and to classify the different genospecies of *B. burgdoferi* sensu lato in ruminant, canine, equine and human population in Pakistan.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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