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.

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).

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.

| District     | Tehsil (prevalence) (%) | Total Positive (%) | 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 (1.2%)    | 5.151 0.02  |
|              | Female 235 (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 |

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.
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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*. Likewise 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 1). 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 1).

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 vegetation and humidity level that help in the life cycle of biological vector. Such factors may be part of the natural cycle in arid and Cholistan areas in Pakistan.

Conclusions

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.

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.

References

Abdalla, M.M., 2007. *Studies on ticks and tick borne diseases of cattle in south Darfur state, Sudan*. MVSc thesis, Department of parasitology, University of Khartoum.

Barbour, A.G., Adrienne, D.P.D. and Jonas, B., 2005. *Infect. Immun.*., 73: 6165–6168. https://doi.org/10.1128/IAI.73.9.6165-6168.2005

Barghash, S., Hahef, A.A., Darwish, A.M. and El-Naga, T.R.A., 2016. *J. Bact. Parasitol.*, 7: 2-7.

Beek, J.V., Sajanti, E., Helve, O., Ollgren, J., Virtanen, M.J., Rissangan, H., Lyytikainen, O., Hytonen, J.
and Sane, J., 2018. *Ticks Tickborne Dis.*, 9: 275-280. https://doi.org/10.1016/j.ttbdis.2017.10.018

Bennet, L., Stjernberg, L. and Berglund, J., 2007. *Vector Borne Zoo. Dis.*, 7: 34-41. https://doi.org/10.1089/vbz.2006.0533

Dehnert, M., Fingerle, V., Klier, C., Talaska, T., Schlaud, M., Krause, G., Wilking, H. and Poggensee, G., 2012. *PLoS One*, 7: 413-421. https://doi.org/10.1371/journal.pone.0041321

Elhelw, R., Elhariri, M., Hamza, D., Abuowarda, M., Ismael, E. and Farag, H., 2021. *BMC Vet. Res.*, 17: 2-9. https://doi.org/10.1186/s12917-020-02733-5

Gasmi, S., Ogden, N.H., Lindsay, L.R., Burns, S., Fleming, S., Badcock, J., Hanan, S., Gaulin, C., Lelamarc, M.A., Russell, C., Nelder, M., Hobbs, L., Graham-Derham, S., Lachance, L., Scott, A.N., Galanis, E. and Koffi, J.K., 2017. *Commun. Dis.*, 43: 194-199. https://doi.org/10.14745/ccdr.v43i10a01

Geebelen, L., Cauteren, D.V., Devleeschauwer, B., Moreels, S., Tersago, K., Oyen, V.H., Speybroeck, N. and Lernout, T., 2019. *Ticks Tick Borne Dis.*, 10: 598-605. https://doi.org/10.1016/j.ttbdis.2018.12.007

Helmy, N., 2000. *J. Egypt. Soc. Parasitol.*, 30: 607-619. https://doi.org/10.1007/BF03022909

Kingry, L.C., Anacker, M., Pritt, B., Bjork, J., Respicio-Kingry, L., Liu, G., Sheldon, S., Boxrud, D., Strain, A. and Oatman, S.D., 2018. *Clin. Infect. Dis.*, 66: 1864-1871. https://doi.org/10.1093/cid/cix1107

Labrini, V., Athanasiou, Victoria, M.S., Eleni, G.K. and Panagiotis, D.K., 2021. *Pathogens*, 10: 2-14.

Liu, R., Wen, Z., Wang, J., Ge, J., Chen, H. and Bu, Z., 2015. *Emerg. Microb. Infect.*, 4: 73-83.

Luo, X.P., 2012. *Inner Mong. Agric. Univ.*, 6: 53-61.

Maraspin, V., Petra, B., Katarina, O., Tereza, R., Eva, R., Andrej, K., Klemen, S., Gary, P.W. and Franc, S., 2021. *J. clin. Med.*, 10: 2-8.

Mohammadpour, R., Mohsen, C., Fateh, T. and Ehsan, M., 2020. *Vet. med. Sci.*, 3: 359-381. https://doi.org/10.1002/vms.239

Obaidat, M.M., Alshehabat, M.A., Hayajneh, W.A. and Roess, A.A., 2020. *Comp. Immunol. Microbiol. Infect. Dis.*, 73: 1-22. https://doi.org/10.1016/j.cimid.2020.101559

Pasha, R.H., Qureshi, A.S. and Khamsa, W.A., 2013. *Int. J. Agric. Biol.*, 15: 62–68.

Praharaj, S.C.A., Jetley, L.C.S. and Kalghatgi, C.A., 2008. *Med J. Armed Forces India*, 64: 26-28. https://doi.org/10.1016/S0377-1237(08)80140-2

Said, M.B., Hanene, B., Alberto, A., Khaoula, A., Manel, Z., Monia, D.J. and Lilia, M., 2016. *Ann. Agric. Environ. Med.*, 3: 442-447.

Samir, A., Ahmed, F., Essam, H.M. and Ahmed, O., 2015. *Trans. Biomed.*, 2: 1-4.

Schwartz, A.M., Hinckley, A.F., Mead, P.S., Hook, S.A. and Kugeler, K.J., 2017. *MMWR Surveill Summ.*, 66: 1-12. https://doi.org/10.15585/mmwr.ss6622a1

Stefancikova, A., Stepanova, G., Derdakova, M., Petko, B., Kyselova, J., Ciganek, J., Strojny, L., Cislakova, L. and Travnicek, M., 2002. *Ticks Tick Borne Dis.*, 5: 219-224. https://doi.org/10.1016/j.ttbdis.2013.10.010

Stoebel, K., Schoenberg, A. and Streich, W.J., 2003. *Epidemiol. Infect.*, 131: 975–983. https://doi.org/10.1017/S0950268803008896

Travniczek, M., Stefancikova, A. and Nadzamova, D., 2002. *Annls Agric. environ. Med.*, 9: 153–155.

Tulloch, J.S.P., Decraene, V., Christley, R.M., Radford, A.D., Warner, J.C. and Vivancos, R., 2019. *BMC Publ. Hlth.*, 19: 931-937. https://doi.org/10.1186/s12889-019-7245-8

WHO, 2020. *Lyme borreliosis (Lyme disease).* Available online: https://www.who.int/ith/diseases/lyme/en/ (accessed on 29 December 2020).

Wilking, H. and Stark, K., 2009. *Ticks Tick Borne Dis.*, 5: 219-224. https://doi.org/10.1016/j.ttbdis.2013.10.010

Yang, J., Liu, Z., Guan, G., Li, Y., Chen, Z., Ma, M., Liu, A., Ren, Q., Wang, J., Luo, J. and Yin, H., 2015. *Annls Agric. environ. Med.*, 22: 208-211. https://doi.org/10.5604/12321966.1152066

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