Research Article

Seroprevalence of Brucellosis in Camels in District Sibi, Balochistan

Hamidur Rahman[1*], Asghar Ali Kamboh[1], Nazar Ali Korejo[2], Atta Muhammad Memon[3], Rashid Ali Korejo[3], Manzoor Ahmed Chandio[1]

[1]Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan; [2]Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan; [3]Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Pakistan.

Abstract | The present study was conducted to find out the seroprevalence of brucellosis in camels of Sibi, Pakistan. A total of 100 serum samples, 50 each from male and female camels were collected from district Sibi, Pakistan and analyzed by competitive enzyme linked immunosorbent assay (cELISA), and rose Bengal plate test (RBPT). The overall seroprevalence of brucellosis in camels was observed as 13% and 17% by RBPT and cELISA respectively. The higher prevalence ($p < 0.05$) found in females (16% and 22%) as compared to males (10% and 12%) by RBPT and cELISA respectively. Camels under 9 years of age, showed relatively higher ($p < 0.05$) prevalence (15.3% and 20%), as compared to camels above 9 years of age (8.5% and 11.4%) through RBPT and cELISA respectively. Breed-wise analysis exhibited that 8% and 16% in Lassi breed, 23%, 30% in Kachhai, 10%, 5% in Kharani and 8%, 8% in Brahvi were found positive through RBPT and cELISA respectively. Likewise, area-wise investigation showed that 17.1% and 22.8% in Talli, 13% and 20% in Kurak, 10% and 10% in Khajjak, and 6.6% and 6.6% in Dephal areas were found positive through RBPT and cELISA respectively. Statistical analysis exhibited the breed ($p < 0.01$) and area ($p < 0.05$) as risk factor for prevalence of brucellosis in camel. Moreover, out of 30 milk samples collected, milk ring test showed 6.6% prevalence. In conclusion, the brucellosis is prevailing in camels of district Sibi. The prevalence is comparatively higher in younger camels as well as in she-camels than older and males respectively.

Keywords | Brucellosis, Camel, cELISA, RBPT, MRT, Pakistan

Received | September 17, 2021; **Accepted** | March 25, 2022; **Published** | June 01, 2022

***Correspondence** | Hamidur Rahman, Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan; **Email**: hamidur408@gmail.com

Citation | Rahman H, Kamboh AA, Korejo NA, Memon AM, Korejo RA, Chandio MA (2022). Seroprevalence of brucellosis in camels in district sibi, balochistan. J. Anim. Health Prod. 10(2): 153-157.

DOI | http://dx.doi.org/10.17582/journal.jahp/2022/10.2.153.157

ISSN | 2308-2801

INTRODUCTION

Camel belong to the family *Camelidae*, order *Artiodactyla* and suborder *Tylopoda* having pad-footed animals (Housawi et al., 2015). Two genera included in the family *Camelidae* are the old phrase genus which include species *Camelus dromedarius* (dromedary, one-humped) and *Camelus bacrianus* (Bactrian, two-humped) (Ali et al., 2016). Camels are comparatively susceptible to many infections like mastitis, Q fever, PPR, leptospirosis, *Chlamydia* infection, *Mycoplasma* infection, tetanus, anthrax, botulism, Johne's disease and tuberculosis. Moreover, certain diseases such as brucellosis, enterotoxaemia, paratuberculosis, and pox virus infection, the camel have more susceptibility than other livestock in the same ecozones (Abbas and Omer, 2005; Gaddafi et al., 2020).

Brucellosis is an ancient bacterial disease of livestock animals with significant zoonotic potential (Jamil et al., 2021).

It is caused by the bacteria belongs to the genus *Brucella* which may transmit vertically or horizontally by several means like sexual contacts, body secretions and licking the aborted fetuses (Pedro et al., 1968). In infected animals, it causes abortion, low rate of fertility and reduced milk production (Hegazy et al., 2011). Due to its zoonotic characteristic it enforces a vast global problem on human health and animal productivity (WHO, 2005). Camels are not the main host for *Brucella*, but are predisposed to two species comprising *B. abortus* and *B. melitensis* (Mangi et al., 2015). Intake of milk and meat from infected camel, especially in nomadic regions where people trust that usage of unpasteurized (without boiling) milk is very effective inside the treatment for diseases, tends in human brucellosis (Garcell et al., 2016). The epidemiology of brucellosis in livestock in different geographical areas has been investigated considerably. Despite its critical significance, research of brucellosis in camels is very limited in many parts of globe including Pakistan (Gul et al., 2015; Baloch et al., 2017). In spite of its demographic and socioeconomic significance, data concerning camel brucellosis rests revealing and limited to other farm animals in the country (Abubakar et al., 2010; Gul et al., 2007). For exact diagnosis serological tests like Rose Bengal plate test (RBPT) is economical and informal for herd based screening of animals with high sensitivity and low specificity (Ali et al., 2013; OIE, 2012). Validation of seropositive animals by more definite antigen based tests, like indirect-ELISA and polymerase chain reaction (PCR) are supportive in deducing diagnosis more exactly (Khan et al., 2020; Khan et al., 2021). Therefore, the present study was intended to highlight the seroprevalence of brucellosis in camels in district Sibi, which would be very helpful for scheming actual control strategies.

MATERIALS AND METHODS

Collection of samples

The samples were randomly collected from camels by using a proforma. A total of one hundred (100) blood samples (10 ml) from two age groups viz; A (<9 years) and B (≥9years) of either sex of camels in Talli, Kurak, Khajjak, and Dephal regions of district Sibi were collected. These samples were collected from Brahvi, Kachhai, Kharani and Lassi breeds of camel through jugular vein by using a plain syringe. Additionally, 30 milk samples were also collected from she camels. All the samples were collected in clean screw-caped plastic cryo-vials and kept these vials in ice box and dispatched to laboratory for detection of *Brucella* species. The samples were centrifuged at 200 g for 15 minutes to obtain sera that were stored at -20 °C until analyzed (Baloch et al., 2017). The study procedures were adopted according to International Animal Ethics guidelines.

Detection of brucellosis by Rose Bengal Plate Test (RBPT)

The serum samples (n=100) were tested by Rose Bengal plate test (RBPT) against the *Brucella melitensis* and *Brucella abort*us antigens (Veterinary Research Institute, Lahore, Pakistan) according to procedure (Falade, 1983). The positive interaction between antigen and serum indicates the appearance of granules, difference in intensity showing the amount of antibodies in the serum of the animal infected with specific species of bacteria. The result of each well was matched with positive and negative controls in the same plate before any conclusions were made.

Detection of brucellosis in camels by Competitive Enzyme Linked Immunosorbent Assay (cELISA)

The (n=100) serum samples collected from camels were tested by cELISA for detection of *Brucella* antibodies using a commercial kit (IDVET, France). The results were read through ELISA reader machine at wavelength of 490nm (Mangi et al., 2015).

Milk Ring Test

The milk (n=30) samples were tested by milk ring test (MRT), as per guidelines of OIE (2009). MRT was performed on individual milk samples. Antigen and milk samples were brought to a room temperature prior to performing the test. About 50µl of antigen was added to 2 ml of milk in a narrow test tube and mixed thoroughly. The tubes then were incubated at 37°C for one hour together with positive and negative working standards. A strongly positive reaction was indicated by formation of dark pink ring above a white milk column. The test was considered to be negative if there was uniform pink color for the milk column and cream layer (Mangi et al., 2015).

Statistical analysis

The JMP 5.0.1a statistical software (SAS Institute Inc., Cary, NC) was used to perform the statistical analysis. Chi-square test was used to find the various risk factors (age, sex, breed and area) for camel brucellosis in the study area. The 95% confidence intervals (CI) was calculated according to method of Dohoo et al. (2010) using t-distribution. The brucellosis prevalence was considered significant at $p < 0.05$

RESULTS

Seroprevalence of brucellosis in camels

In this study the seroprevalence of brucellosis in camels was detected by RPBT and cELISA. The highest prevalence (17%) was detected through cELISA followed by 13% through RBPT as shown in Table-1. The statistical analysis exhibited a significant ($p < 0.05$) difference between cELISA and RBPT results. In RBPT, all positive samples

Table 1: Seroprevalence of brucellosis in camels analyzed by RBPT and cELISA in district Sibi

Test*	Total No. of samples	No. of positive samples	Prevalence percentage
RBPT	100	13	13%
cELISA	100	17	17%

* RBPT: Rose Bengal plate test; cELISA: Competitive enzyme linked immunosorbent assay.

Table 2: Seroprevalence of brucellosis in camels in relation to various risk factors

Variable	Categories	RBPT*				cELISA*			
		Total No. of samples	No. of positive samples (prevalence)	95% CI**	*p*-value	Total No. of samples	No. of positive samples (prevalence)	95% CI**	*p*-value
Age	<9 year	65	10 (15.3%)	12.3-16.9	0.001	65	13 (20%)	17.6-24.5	0.009
	≥9 year	35	3 (8.5%)	6.4-10.1		35	4 (11.4%)	9.9-12.4	
Sex	Male	50	5 (10%)	8.5-12.7	0.026	50	6 (12%)	10.0-15.1	0.000
	Female	50	8 (16%)	13.8-17.9		50	11 (22%)	19.4-26.2	
Breed	Lassi	25	2 (8%)	6.3-9.4	0.010	25	4 (16%)	14.2-18.7	0.000
	Kachhai	30	7 (23%)	20.2-25.0		30	9 (30%)	26.3-33.6	
	Kharani	20	2 (10%)	9.1-12.0		20	1 (5%)	4.4-5.7	
	Brahvi	25	2 (8%)	7.1-9.6		25	2 (8%)	7.0-9.2	
Area	Talli	35	6 (17.1%)	15.3-18.9	0.045	35	8 (22.8%)	19.8-25.2	0.038
	Kurak	30	4 (13.3%)	11.7-15.1		30	6 (20%)	17.9-21.8	
	Khajjak	20	2 (10%)	8.7-11.9		20	2 (10%)	9.2-11.4	
	Dephal	15	1 (6.6%)	6.1-7.4		15	1 (6.6%)	5.8-7.5	

* RBPT: Rose Bengal plate test; cELISA: Competitive enzyme linked immunosorbent assay.
** CI: confidence interval

(n=13) were reacted against *B. abortus* antigen and none of the sample was found positive against *B. melitensis* antigen.

Seroprevalence of brucellosis in camels in relation to various risk factors

The results presented in Table-2 shows prevalence of brucellosis in relation to various risk factors in camels of the study area. A comparatively higher ($p < 0.01$) prevalence was detected in young camels (<9 years) i.e., 15.3% and 20% through RBPT and cELISA respectively as compared to older (≥9 years) i.e., 8.5% and 11.4% detected through RBPT and cELISA respectively. The higher ($p < 0.05$) prevalence 16% and 22% were found in she camel through RBPT and cELISA respectively, while 10% and 12% prevalence were found in male camels through RBPT and cELISA respectively (Table 2).

The higher prevalence 23% and 30% were found in Kachhai breed through RBPT and cELISA respectively. Lassi breed have the prevalence of 8% and 16%, Kharani breed have prevalence of 10% and 5%, and Brahvi breed have 8% and 8% prevalence found through RPBT and cELISA respectively. The statistical analysis exhibited the breed ($p < 0.01$) as risk factor for prevalence of brucellosis in camels of district Sibi (Table 2).

A total of 35, 30, 20 and 15 samples from Talli, Kurak, Khajjak and Dephal were tested through RBPT and cELISA respectively. The result shows prevalence of 17.1% and 22.8% in Talli area, 13.3% and 20% in Kurak, 10% and 10% in Khajjak area, and 6.6% and 6.6% in Dephal area, tested through RBPT and cELISA respectively. The statistical analysis exhibited the area ($p < 0.05$) as risk factor for prevalence of brucellosis in camels of district Sibi (Table 2).

Detection of *Brucella* species in milk samples collected from camels

In the present investigation 30 milk samples were also tested for presence of *Brucella* species in milk through MRT. The results demonstrated that out of 30 samples, 2 samples were positive and the positive prevalence rate was 6.6% (data not shown).

DISCUSSIONS

During present study, out of 100 serum samples examined by RBPT and cELISA, 13 and 17 sera were found positive for brucellosis respectively. These results are in accordance with Junaidu et al. (2006) who reported 13 and 21% by RBPT and cELISA respectively. While in another study, the overall seroprevalence of brucellosis in camels

was recorded 19.4% by RBPT (Mukhtar et al., 2007). Furthermore, our results demonstrated that all sera samples were found negative for *B. melitensis* antibodies. The study of Khan et al. (2020) reported that out of 32 camel samples, 25 were positive for *B. abortus*, 5 were positive for *B. suis* and 2 were found positive for *B. melitensis* using real-time PCR. The study concluded that *B. melitensis* infection in camels is rare and probably have in those herds only, which had mixed rearing with sheep/goat. This theory is also supported by the previous workers who worked on the role of small ruminants in transmission of brucellosis (Abdel-Hamid et al., 2017).

This study found cELISA more suitable technique for detecting brucellosis in camels as compared to RBPT because of its' higher sensitivity. In line, Khan et al. (2020) also found higher detection of *Brucella* antibodies in camel samples by cELISA (20.2%) as compared to RBPT (15.5%). The present studies also investigated the gender vise seroprevalence of brucellosis in camels. The higher prevalence of brucellosis was detected in she camels compared with male even on both techniques i.e. cELISA and RBPT. The gender vise screening of the camels for seroprevalence of brucellosis suggested that the she-camels are at high risk of infection than the male. Concomitantly, a higher seroprevalence (38.5%) of brucellosis was observed in adult she camels which had history of reproductive problems such as abortion, still birth and retained placenta, during a cross-sectional study in Dire Dawa, Ethiopia (Ismail et al., 2012). These results are also in agreement with the study of Shahzad et al. (2017) who reported 4.46 and 1.21% prevalence in female and male camels respectively analyzed by RBPT. Contrary to this, the study of Khan et al. (2020) reported a higher seroprevalence of *Brucella* antibodies in male camels than she-camels using four different serological techniques.

Moreover, the seroprevalence of brucellosis in different age groups of camels were also investigated. The greater (20%) sero-positive cases of brucellosis were determined in the camels that were under 9 years of the age, however, the animals above age of the 10 years showed only 11.4% progression towards the disease. The reason of higher prevalence of brucellosis infection in camels less than 9 years of the age might be due to lesser immunity/resistance of the animals. Similarly, 12.4% prevalence of brucellosis was reported in camels with age varied from the 5.5-10 years (Junaidu et al., 2006). While, animals over 5 years of the age have been reported with 3.98% prevalence of brucellosis (Abou-Eisha 2000). Likewise, in another study 22.9 and 27.9% seroprevalence was recorded in camels of <8 years, and ≥8–11 years as compared to 13.7% that recorded in age group of 11-13 years (Khan et al., 2020)

CONCLUSION

This study concluded that the brucellosis is prevailing in different species of the camels of district Sibi, Balochistan and high prevalence of the infection was observed in the she-camels and young animals (less than 9 years of the age). Further studies warranted to assess the various *Brucella* species as well as genotype and biovar identification in camels using molecular tools.

ACKNOWLEDGEMENTS

The first author (HR) is highly thankful to field colleagues/ veterinarians for their keen support and help in sample collection.

NOVELTY STATEMENT

Current study is a first report that have highlighted the status of camel brucellosis in District Sibi of Balochistan using the serum based techniques i.e., RBPT and cELISA. The study have also illuminated the association of various risk factors with the seroprevaelnce of brucellosis in camels of the study area.

AUTHORS CONTRIBUTION

HR collected the samples and carried out analysis in the lab. AAK planned the study and proof read the manuscript. NAK and AMM helped in statistical analysis as well as in manuscript writing. RAK helped in final drafting and revisions of manuscript. MAC assist the HR in sample collection and laboratory experiments.

REFERENCES

Abass AH, Mithal K, Aj Al. Gharban H, Foad M., Luma (2018). Application of three Diagnostic Serologic Techniques to Detect of Dromedary Camel's Brucellosis. Al-Qadisiyah J. Pure Sci. 23(2): 61 - 74. https://doi.org/10.29350/jops.2018.23.2.738

Abbas B, Omer OH (2005). Review of infectious diseases of the camel. Vet. Bulletin. 75(8): 1 – 16.

Abdel-Hamid NH, Elbauomy EM, Ghobashy HM, Sayour AE, Ismail RI, Soliman HS, Abdel-Haleem MH (2017). Role of sheep and goat mobile flocks in the transmission of brucellosis to the household ruminants and the disease prevalence in these flocks. Anim. Health Res. J. 5: 95–105.

Abou-Eisha AM (2000). Brucellosis in camels and its relation to public health. Assiut. Vet. Med. J., 44, 87: 54-64. https://doi.org/10.21608/avmj.2000.182064

Abubakar M, Arshed MJ, Hussain M, Ehtisham-ul-Haq, Ali Q (2010). Serological evidence of *Brucella abortus* prevalence in Punjab province, Pakistan a cross-sectional study. Transboundry Emer. Dis. 57(6): 443-447. https://doi.

org/10.1111/j.1865-1682.2010.01171.x

Ali F, Hussain R, Qayyum A, Gul ST, Iqbal Z, Hassan MF (2016). Milk somatic cell counts and some hemato-biochemical changes in sub-clinical mastitis dromedary she-camels (Camelus dromedaries). Pak. Vet. J. 36(4): 405-408.

Ali S, Ali Q, Abatih EN, Ullah Ali, M, Khan I, Akhter S (2013). Sero-prevalence of *Brucella abortus* among dairy cattle and buffalo in Pothohar Plateau, Pakistan. Pakistan J. Zool., 45: 1041-1046.

Baloch AS, Rasheed A, Rind R, Sahito JK, Buriro R, Ayoob MF, Dewani P (2017). Seroprevalence of Brucellosis in camels in Sindh, Pakistan. Pakistan J. Zool. 49(1). https://doi.org/10.17582/journal.pjz/2017.49.1.sc5

Dohoo I, Martin W, Stryhn H (2010). Veterinary epidemiologic research, 2nd edn. VER Inc., Charlottetown, Canada

Falade S (1983). Some observations in the use of Rose Bengal plate and tube agglutination in caprine brucellosis. Trop. Vet. 1: 49-53.

Gaddafi MS, Faleke OO, Yakubu Y, Garba B, Musawa IA, Junaidu AU, Magaji AA, Alkali BR, Aliyu MA (2020). Seroprevalence of the middle east respiratory syndrome coronavirus antibodies in camels from two international animal control-posts, Kebbi State, Nigeria. J. Anim. Health Prod, 8(2): 50-54.

Garcell HG, Garcia EG, Pueyo PV, Martín IR, Arias AV, Serrano RNA (2016). Outbreaks of brucellosis related to the consumption of unpasteurized camel milk. J. Infect. Pub. Health. 9(4): 523-527. https://doi.org/10.1016/j.jiph.2015.12.006

Ghanem YM, El-Khodery A, Saad AA, Abdelkader AH, Heybe A, Musse YA (2009).Seroprevalence of camel brucellosis (Camelus dromedarius) in Somaliland. Trop. Anim. Health Prod`. 41(8): 1779-1786. https://doi.org/10.1007/s11250-009-9377-9

Gul ST, Khan A, Ahmad M, Rizvi F, Shahzad A, Hussain I (2015). Epidemiology of brucellosis at different livestock farms in the Punjab, Pakistan. Pak. Vet. J. 35(3): 309-314.

Gul S, Khan A. (2007). Epidemiology and epizootology of brucellosis: a review. Pak. Vet. J. 27(3): 145-151.

Hegazy YM, Moawad A, Osman S, Ridler A, Guitian J (2011). PLoS Negl. Trop. Dis., 5: e944. https://doi.org/10.1371/journal.pntd.0000944

Housawi FMT, Zaghawa AA, Al-Naeem A. (2015). Seroprevalence of paratuberculosis among camels in Al-Ahsa and Riyadh regions, Kingdom of Saudi Arabia. Pak. Vet. J. 35(3): 375-378.

Ismail WSA, Temesgen W, Molla W (2012). Seroprevalence and associated risk factors of camel (Camelus dromedaries) brucellosis in and around Dire Dawa, Ethiopia, Global Veterinaria, 8 (5): 480-483.

Jamil T, Khan AU, Saqib M, Hussain MH, Melzer F, Rehman A, Shabbir MZ, Khan MA, Ali S, Shahzad A, Khan I (2021). Animal and Human Brucellosis in Pakistan. Frontiers in Public Health, 9. https://doi.org/10.3389/fpubh.2021.660508

Junaidu AU, Oboegbulem SI, Sharubutu GH, Daneji AI (2006). Brucellosis in camels (Camelus dromedaries) slaughtered in Sokoto, northwestern Nigeria. Anim. Prod. Res. Adv., 2 (3): 158-160. https://doi.org/10.4314/apra.v2i3.36330

Khan AU, Sayour AE, Melzer F, El-Soally S.A.G.E., Elschner MC, Shell WS, Moawad AA, Mohamed SA, Hendam A, Roesler U, Neubauer H (2020). Seroprevalence and molecular identification of Brucella spp. in camels in Egypt. Microorganisms., 8(7): 1035. https://doi.org/10.3390/microorganisms8071035

Khan I, Ali S, Hussain R, Raza A, Younus M, Khan N, Faheem M (2021). Serosurvey and potential risk factors of brucellosis in dairy cattle in peri-urban production system in Punjab, Pakistan. Pak Vet. J. 10.

Mangi MH, Kamboh AA, Rind R, Dewani P, Nizamani ZA, Mangi AR, Nizamani AR, Vistro WA (2015). Seroprevalence of brucellosis in Holstein-Friesian and indigenous cattle breeds of Sindh Province. Pakistan. J. Anim. Health Prod., 3(4): pp.82-87. https://doi.org/10.14737/journal.jahp/2015/3.4.82.87

Mukhtar MO, Sarah AA, Adusalam MA, Ahmed AM (2007). Survey of brucellosis among sheep, goats, camels and cattle in Kassala area, Eastern Sudan. J. Anim. Vet. Adv., 6.5: 635-637

OIE (2012). Bovine Brucellosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Paris, Vol-I: 01-35.

OIE, (2009). Office Internationale des Epizooties. Manual of Standards for Diagnostic Tests and Vaccines, 6th ed. France, OIE Press, pp: 389-428.

Pedro-Pons A, Farreras P, Foz A, Surós J, Surinyach R, Frouchtman R (1968). Patol.Clín. Méd., Vol. VI. Salvat Ediciones S.A. Madrid.

Shahzad A, Khan A, Khan MZ, Saqib M (2017). Seroprevalence and molecular investigation of brucellosis in camels of selected districts of Punjab, Pakistan. The Thai J. Vet. Med. 47(2):.207.

World Health Organization, (2005). The control of neglected zoonotic diseases. Report of a joint WHO/DFID-AHP. Geneva: pp. 54.