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Seroprevalence and molecular investigation of brucellosis in camels of selected districts of Punjab, Pakistan

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Original Article

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Abstract

Camels still play an important role in providing food security in several developing countries, especially in the arid zones. As brucellosis has zoonotic importance, thereby diseased camels can infect humans chiefly via milk. In spite of its vital importance, limited studies are available of brucellosis in camels in Pakistan. Keeping in view the fact, this study was designed and executed. For this purpose, serum samples (n=761) from various herds of camel with history of abortion from different locations of Punjab province, Pakistan were collected. Initial screening was done using Rose Bengal plate test (RBPT). To avoid improper judgment of the diagnosis of brucellosis caused by serological tests, positive samples were subjected to competitive enzyme-linked immunosorbent assay (cELISA). Detection of *Brucella* genome was carried out through conventional PCR in RBPT and cELISA positive test samples. Overall, 3.41% (n=26, 95% CI 2.24-4.97) of the camels were seropositive with RBPT. Prevalence of brucellosis significantly varied in the animals with different geographical source, sex, age, lactation number and health status. In molecular analysis, out of 18 samples confirmed through cELISA, only two samples were positive for *Brucella* genome through PCR. The sequences were aligned with reported sequences in NCBI GenBank and revealed 100% sequence homology with the *bcsp-31* gene of *Brucella* reported from other parts of the world. The study highlights the epidemiologic, economic and public health impact of camel brucellosis as a basis for designing further diagnostic improvements and effective control strategies.

Keywords: Brucellosis, camel, epidemiology, seroprevalence

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Introduction

Camelids belong to the family Camelidae, order Artiodactyla and suborder Tylopoda having pad-footed animals (Schwartz and Dioli, 1992; Housawi et al., 2015). Two genera included in the family Camelidae are the old-world genus including species Camelus dromedarius (dromedary, one-humped or Arabian camel) and Camelus bactrianus (bactrian or the two-humped camel); and the new world camels including species Lama glama, Lama guanicoe, Lama pacos and Vicugna vicugna (Abbas and Agab, 2002; Ali et al., 2016). Dromedary camels are found in the dry and hot areas of west central Asia, North Africa, the Near East and Ethiopia. The two humped camel inhabits the cold deserts of southern areas of the former Soviet Union, Mongolia, East-Central Asia and China (Wilson, 1984; Alsobayil et al., 2015).

In the arid zones of Asia camels share a major role in the survival of millions of people because of its socioeconomic importance (Sprague et al., 2012). The ability of camel to not only survive severe droughts but also produce milk and continue reproducing makes it fit domestic animal in severe drought (Schwartz, 1992). Many communities depend on camels for their living and income throughout the world. The reliance on camel consists of consumption of their meat and milk, utilization of their leather and wool, exportation of live camels, use of camel as animal for transport, packing and riding and sport and tourism resource (Kohler-Rollefson, 2000). Camels were considered as resistant animals to many disease factors for a long time in the past. Nevertheless, under natural habitat they are exposed to severe stress factors which make them vulnerable to disease conditions like other livestock (Abbas and Tilley, 1990; Abbas and Agab, 2002).

Brucellosis is a serious dreadful zoonotic ailment affecting all domesticated animals including camels (Radostits et al., 2007; Gwida et al., 2012). Nevertheless, brucellosis in camel has not received any proper attention from researchers. Brucellosis has been reported in many camel keeping countries (Wernery, 2014). Camels are not the primary host for *Brucella*, but are susceptible to two species including *B. abortus* and *B. melitensis* (Zhang et al., 2015). Accordingly, the contact of camel with the primary hosts of the disease measures the infection and prevalence rate in the camel. Consumption of milk and meat from diseased camel, especially in nomadic areas where people believe that utilization of raw milk is very effective in the cure for ailments, leads to human brucellosis (Al Tawfiq and Abukhamsin, 2009; Abu-Seida et al., 2015; Garcell et al., 2016).

The epidemiology of brucellosis in cattle and small ruminants in different geographical areas has been investigated extensively. In spite of its vital importance, studies of brucellosis in camels are very limited in Pakistan (Ajmal et al., 1989; Nasrin et al., 1998; Gul et al., 2014; Gul et al., 2015). Moreover, previous studies are based on serological investigations and data are scanty on molecular investigations into camel brucellosis in Pakistan. Therefore, the present study was designed to highlight the seroprevalence of brucellosis and molecular detection of brucellosis in camel, which would be very helpful for designing effective control strategies.

Materials and Methods

Study localities and sampling: This study was executed in accordance with all national legislation concerning the protection of animal welfare and followed guidelines set by the Graduate Studies and Research Board (GSRB), University of Agriculture, Faisalabad, Pakistan. The study's protocol was approved by GSRB vide letter No. DGS/42329-32; Dated: 14-10-15. This study was conducted in six southern and central districts of Punjab province including Faisalabad, Jhang, Muzafargarh, Bhakkar, Layyah and Bahawalpur (Fig. 1). Since the primary objective of the study was to detect *Brucella* species involved in the causation of disease in camels, an inclusive protocol was followed to collect samples from herds with history of recent abortion. To this end, blood samples (n=761) were collected. Both animal and herd level information was registered on a structured questionnaire. Important information including location, type (single/mixed), size of herd, vaccination history and methods of disposal of abortive fetuses at farm was recorded. Significant animal level data including age, breed, breeding method, pregnancy and lactation status, history of abortion, retained placenta or other reproductive disorders were also recorded. The camels were divided into four age groups (<1 year, 1 to <3 years, 3 to <7 years, >7 years).

The blood samples were collected in gel and clot activator vacutainers (Xinle®, China) and were subjected to centrifugation (5000 rpm for 8 min) for serum collection. The serum samples were preserved at -40°C until further testing.

Rose Bengal plate agglutination test: All serum samples were initially screened for presence of antibodies contrary to antigen of *Brucella* by the Rose Bengal plate agglutination test (RBPT). The test was performed on a glass plate using the antigens procured from Veterinary Research Institute, Lahore, Pakistan. Both antigen and sera were settled at room temperature before testing. On the test plate, both antigen and serum in equal volume (25 µL) were placed and mixed by a stirrer. Then the plate was shaken for 3 min and the test sample was observed for agglutination (OIE, 2008).

Competitive enzyme-linked immunosorbent assay (cELISA): Doubtful or positive samples with RBPT were further confirmed by cELISA (Savanova®, Sweden, Germany) according to the manufacturer's instructions. Percent inhibition (PI) for determining seropositivity of the tested samples was calculated as ≥30% of the mean of Optical Density (OD) of the 2 conjugates control as:

$$\text{PI} = \frac{(mean\ OD\ sample * 100)}{mean\ OD\ congugate\ control}$$

***Genomic studies*:** DNA was extracted from the serum samples positive for RBPT and cELISA using a commercial DNA extraction kit (Favorgen®, FABGK 001) according to the manufacturer's protocol. Gene amplification was performed in a thermal cycler

(BioRad®, T100™ Thermal Cycler) using primers B4-F (5′TGGCTCGGTTGCCAATATCAA3′) and B5-R (5′CGCGCTTGCCTTTCAGGT CTG3′) of gene of BCSP31 protein, giving a final product of 223 bp (Baily et al., 1992). The amplified product was analyzed on 1.5% agarose gel and visualized under UV light in gel documentation system (BioRad®, Gel Doc™ EZ Gel Documentation System). DNA sequencing of positive amplicon was performed through commercial sequencing services (Macrogen®, Korea). Sequence analysis was carried out for confirmation and to detect inter-organism homology.

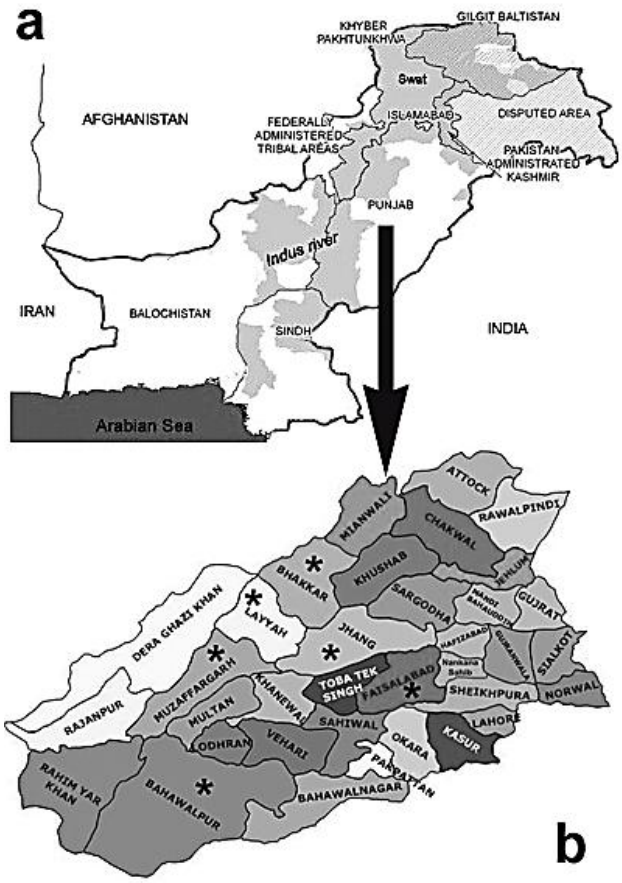

Figure 1 a) Maps of Pakistan and its surrounding neighbors, and b) Asterisks indicate the southern and central districts of Punjab where samples were collected.

Data analysis: The Chi-square test for independence was used to determine if the prevalence varied between genders, age groups, locations, lactation number and pregnancy status. The Fisher's exact test was used if any of the cells were less than 5 in a 2 x 2 table. Confidence intervals (CI) were calculated using the Exact Binomial Method for prevalence estimates. Odds ratios (OR) along with 95% CI were derived to determine associations between factors and presence of antibodies to brucellosis. Associations between outcome response variables (seropositivity) and explanatory variables (information recorded through the Proforma) were estimated using binary logistic regression (MINITAB 16 for Windows®).

Results

RBPT-based seroprevalence of brucellosis in camels: The prevalence of brucellosis was determined through RBPT in relation to different factors which were geographical source, sex, age, pregnancy status, lactation number and health status. Overall, 3.41% (n=26; 95% CI: 2.24-4.97) of the camels were seropositive with RBPT (Table 1). The serum samples positive through RBPT (n=26) were further confirmed by cELISA, which showed 18 to be seropositive for *Brucella* antibodies.

The positivity of *Brucella* antibodies region-wise checked by RBPT differed significantly (χ^2=23.35; df=5; P=0.00) in different districts. The highest positivity was observed in Faisalabad district (9.03%) followed by Jhang and Muzaffargarh districts, whereas no positive case of *Brucella* was detected in Bhakar, Layyah and Bahawalpur districts of Punjab, Pakistan. The odds ratio indicated that the prevalence of Brucella was 0.37 and 0.2 times less in Jhang and Muzaffargarh than in Faisalabad (Table 1).

The RBPT-based prevalence of brucellosis in relation to sex was higher in female camels (4.46%) compared to male camels 1.21% (Table 2). The camels were divided into four age groups (<1 year, 1 to <3 years, 3 to <7 years, >7 years). Difference in the occurrence of brucellosis was significant among the different age groups when tested with RBPT (χ^2=10.88, df=3; P=0.01). Through RBPT, prevalence of the disease was the highest (6.98%) in the camels >7 years followed by the camels <1 year (3.27%), 1 to <3 years (2.04%) and 3 to <7 years (1.77%). The logistic regression analysis showed that the disease prevalence probability was 0.61 and 0.53 times less and 3.98 times more in the camels of 1 to <3 years, 3 to <7 years and

>7 years, respectively, compared to the camels <1 year (Table 2).

The RBPT-based prevalence of brucellosis differed significantly (χ^2=3.13, df=2; P=0.01) in the animals with different health condition. The RBPT-based prevalence was the highest (6.67%) in animals having poor health followed by animals with moderate and good health condition. The odds ratios indicated that brucellosis was 0.61 and 0.05 times less in the animals with moderate and good health condition, respectively, compared to the animals with poor health condition (Table 2).

The RBPT-based prevalence of brucellosis in non-pregnant versus pregnant animals was 5.28% versus 2.87% with insignificant (χ^2=1.44, P=0.23) difference. The prevalence of brucellosis was higher in animals with more number of lactation (7.83%) compared to animals with less number of parities (3.57 and 2.53%). The logistic regression analysis of RBPT-based results indicated that the disease prevalence probability was 0.31 and 0.44 times less in animals with 0 and 1 parity number, respectively, compared to animals with 2 or more parity numbers (Table 3).

Table 1 Prevalence of brucellosis in camels in different districts of Punjab, Pakistan through RBPT

Parameter/ Region	Total samples	Positive	Percentage	Coefficient	SE Co-ef	P-value	Odds Ratio	95% CI
Faisalabad	166	15	9.03	-2.31	0.27	0.00	-	5.15-14.47
Jhang	282	10	3.54	-0.99	0.42	0.02	0.37	1.71-6.42
Bhakar	191	0	0	-21.09	5283	0.99	0.00	0.00-1.91
Muzaffargarh	51	1	1.96	-1.60	1.05	0.13	0.20	0.05-10.45
Layyah	20	0	0	-21.09	16324	0.99	0.00	0.00-16.84
Bahawalpur	51	0	0	-21.09	10228	0.99	0.00	0.00-6.98
Total	761	26	3.41					2.24-4.97

Chi-Square=23.35, P-value=0.00, df=5

Table 2 Prevalence of brucellosis assessed through RBPT in relation to sex, age and health status of camels of Punjab, Pakistan

Parameters	Total samples	Positive	Percentage	Coefficient	SE Co-ef	P-value	Odds Ratio	95% CI
Sex								
Male	246	3	1.21	-1.33	0.62	0.031	0.26	0.25-3.52
Female	515	23	4.46	-3.06	0.21	0.00	-	2.85-6.63
	Chi-Square=5.02, P-value=0.03, df=1							
Age (Years)								
<1	61	2	3.27	-3.38	0.72	0.00	-	0.40-11.35
1 to <3	147	3	2.04	-0.49	0.96	0.59	0.61	0.25-3.51
3 to <7	338	6	1.77	-0.63	0.83	0.44	0.53	0.65-3.82
7 to >7	215	15	6.98	0.79	0.77	0.30	2.21	3.96-11.25
	Chi-Square=10.88, P-value=0.01, df=3							
Health Status								
Good	272	1	0.36	-2.96	1.05	0.005	0.05	0.01-2.03
Moderate	309	13	4.20	-0.49	0.41	0.24	0.61	2.26-7.09
Poor	180	12	6.67	-2.63	0.29	0.00	-	3.49-11.36
	Chi-Square=3.13, P-value=0.01, df=2							
Total	761	26	3.41					2.24-4.97

Table 3 Prevalence of brucellosis assessed through RBPT in relation to pregnancy status and parity in camels of Punjab, Pakistan

Parameters	Total samples	Positive	Percentage	Coefficient	SE Co-ef	P-value	Odds Ratio	95% CI
Pregnancy status								
Pregnant	174	5	2.87	-0.63	0.51	0.218	0.53	0.94-6.58
Non-pregnant	341	18	5.28	-2.89	0.24	0.00	-	3.16-8.21
	Chi-Square=1.44, P-value=0.23, df=1							
Parity Number								
0	237	6	2.53	-1.19	0.50	0.02	0.31	0.93-5.43
1	112	4	3.57	-0.83	0.59	0.16	0.44	0.98-8.89
2 or more	166	13	7.83	-2.47	0.29	0.00	-	4.24-13.02
	Chi-Square=6.03, P-value=0.04, df=2							
Total	515	23	4.46					2.85-6.63

Molecular investigation of brucellosis in camel: Molecular identification of the genus *Brucella* was carried out utilizing B4/B5 primer pair for the expected amplified product of 223 bp (for the region of the sequence encoding a 31 kDa periplasmic immunogenic *bcsp*31 gene; Fig. 2). Out of the 18 samples tested positive through cELISA, only 2 samples were found positive for *Brucella*. Dideoxy (Sangar)-based DNA sequencing of the positive samples was analyzed by ChromasPro® (Technelysium Pty Ltd) for detection of any sequencing error. Error-free sequences were further analyzed by DNA data banks available online (GenBank, NCBI), by using BLAST program. Finally, *Brucella* sequence was submitted to GenBank (*Brucella* PAK-CAMEL; Accession No. KX618687).

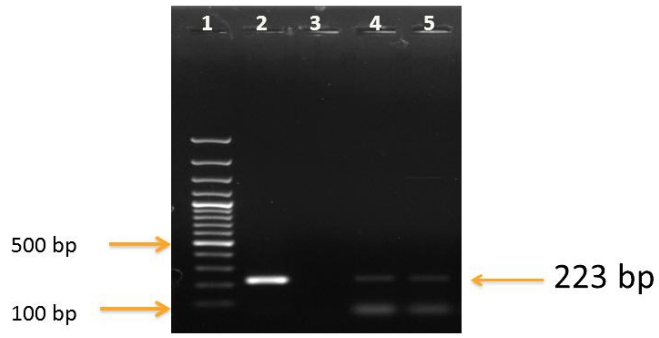

Figure 2 Photograph of selected samples positive for Brucella Lane description is as follows: 1 ladder (Thermo Scientific®, GeneRuler 100 bp Plus), 2 control +ve, 3 control –ve, 4 and 5 positive test samples

Discussion

The camel population of Pakistan is estimated to be approximately one million (Pakistan Economic Survey, 2015). These camels are mostly grazed in the desert by nomads following traditional husbandry methods. Others are raised either under intensive management systems, in small farms, small groups or backyards as an immediate source of meat, milk and drought purpose.

The pervasiveness of brucellosis in all livestock in Pakistan has been reported by various authors. However, at national level a limited number of literature is available on the prevalence rate of the disease in camel. The infection has been confirmed in cattle, buffalo, goats, sheep and humans. The present review data shows that *B. abortus* was responsible for most of the cases of livestock infection.

In the current study, 3.41% of the camels were seropositive with RBPT. Previous studies reported a very high level of the prevalence of brucellosis in camels. However, most of these studies were conducted in high risk population and that is why a higher prevalence rate was reported (Omer et al., 2010; Al-Majali et al., 2008). In Pakistan, few studies regarding brucellosis in camel reported 2.0-3.07% prevalence similar to our study (Gul et al., 2014).

Many factors, including host, agent and environmental factors, directly or indirectly influence the prevalence, distribution and transmission of a disease (Burridge, 1981). A large herd size, high stocking density, older animals, frequent introduction of untested livestock, unrestricted grazing and grazing of communal pastures can all be associated with high seroprevalence of brucellosis (Kadohira et al., 1997; Schelling et al., 2003).

In order to estimate the effects of risk factors on the seroprevalence of disease, several parameters were structured in form of a questionnaire and the results are presented in Tables 1 to 3. Information on disease is often collected from two sources: the owner and direct observation of herds/flocks. However, in Pakistan, the quality of information collected from these sources may be questionable. In the current study, most private livestock owners had no systematic herd records or an animal identification system. Consequently, no reliable data were available regarding the number of births, early mortalities, birth of weak young or stillbirths or number of abortion occurring each year in the flocks/herds. Most of the sampled herds were managed by illiterate expatriate workers who were not familiar with the origin of the animals, whether they had been purchased or not.

Several factors were analyzed as potential risk factors at individual level. In this study the individual animal factors analyzed included geographic source, age, sex, parity, pregnancy status and health status of animals. The questionnaire-based information collected during this study indicated that several factors could be considered as potential risk factors for the disease, increasing the risk of animals infected with brucellosis. However, the risk factors associated with seropositivity varied between species at individual levels in the univariable analyses.

The camels in Faisalabad district were more likely to be seropositive (9.03%) than the camels in the other districts. These results highlight the prevalent nature of brucellosis in central Punjab. This may be attributed to the close contact of livestock species, lack of herd health program, disorganized management system, frequent induction of high yielding animals without quarantine, higher population density of livestock and shared grazing along with poor management practices adopted by farmers in this region (Munir et al., 2011; Sikder et al., 2012). It is well documented that the disease transmit betweeen species (Dawood, 2008) and these findings are in accord with previous reports of higher prevalence levels in camels kept along with large and small ruminants (Abou-Eisha, 2000; Al-Majali et al., 2008).

In the present study, the prevalence of brucellosis was evident in the female animals only. Higher seroprevalence was also evident in female animals in previous studies (Bayemi et al., 2009; Hadush et al., 2013). Females are generally kept for longer period of time than males and this is likely the cause of increased opportunity for exposure to the bacterium (Mekonnen et al., 2010). Relatively higher vulnerability of female animals could also be that females are more physiologically anxious than male animals (Walker, 1999).

Infection may occur in camels of all ages but more persistent in sexually mature animals (Aulakh et al., 2008; Abubakar et al., 2010). The study reveals significant difference between young and adult age groups. Significantly higher prevalence of brucellosis in the adult age groups was observed compared to the lower age groups similar to previous studies (Dawood, 2008; Balcha and Fentie, 2011). Animals are more expected to be exposed to disease when they grow older. With the increase in age, increase in the level of hormones and erythritol of the animals may be attributed to the enhanced growth of *Brucella* (Poester et al., 2013). Similarly, younger animals have a tendency to be resistant to brucellosis and frequently clear infections although latent infections may occur.

Moreover, chances of the occurrence of disease are more in animals which graze freely on contaminated pasture compared to young animals which have not reached reproductive age (Radostits et al., 2007).

In the present investigation, a statistically insignificantly higher seropositivity was recorded in the animals with poor health status followed by moderate and good health status. In other studies, the association between health status and *Brucella* infection in camels has also been described (Musa and Shigidi, 2001).

In the present study, the animals with more parity number had insignificantly higher prevalence rate of brucellosis as in age groups. The animals with more parity numbers were found more likely to be seropositive compared to the animals with less parity number. Similar observations were reported by others (Gul et al., 2014). The present study indicated insignificant difference in the occurrence of the disease in pregnant compared to non-pregnant animals. Exposure seems to be more common in sexually mature animals and the possibility of infection increases with the period of pregnancy (Crawford et al., 1990).

The diagnosis of brucellosis in clinical cases cannot be achieved easily. There are several serological tests available for the diagnosis of brucellosis, but cross reactivity is a major problem. Isolation of the organism is still the gold standard for definitive diagnosis of the disease which is time-consuming and could be hazardous. Moreover, this procedure is laborious and entails a considerable turnover time (~1 week). It also requires a biosafety level 3 laboratory and skilled technical personnel. Handling of live *Brucella* cultures involves high risk of laboratory-acquired infections, therefore, very strict biosafety rules must be observed.

Molecular diagnostic methods (e.g. PCR) have considerably reduced this risk and are the most reliable tools in terms of sensitivity and specificity. More than 400 scientific reports are available on the rapid detection of organism to the differential identification of species and strains of brucellosis (Poester et al., 2010). Although PCR tests have high sensitivity and specificity, serological assays are easier to use and more widely adopted in the field.

Several types of primer pairs have been used to identify the genus *Brucella*. The primer sequences have been derived from polymorphic regions of genomes and include sequences encoding *BCSP-31* (B4/B5) (Baily et al., 1992), 16SrRNA(F4/R2) (Romero et al., 1995), 16S-23S intergenic transcribed spacers (Bru ITS-S/Bru ITS-A) (Rijpens et al., 1996; Bricker, 2002), 16S-23S rDNA interspace (ITS66/ITS279) (Keid et al., 2007), IS711 (IS313/IS639) (Hénault et al., 2000), *per* (*bruc1/bruc5*) (Bogdanovich et al., 2004), *omp2* (*JPF/JPR*) (Leal-Kleveza, 1995), outer membrane proteins (*omp31, omp 2b* and *omp2a*) (Imaoka et al., 2007) and proteins of the *omp31/omp25* family of *Brucella spp.* (Vizcaino et al., 2004). The diagnostic sensitivity and specificity of these sets of primers have been found to be inconsistent. PCR assays targeting the 16S-23S rRNA operon and *Brucella bcsp31* gene are highly conserved in the *Brucella* genus and are often used for the screening of brucellosis in humans, animals and food samples (e.g. serum, blood and milk) (Bricker, 2002). Comparative analyses of three genus-specific PCR assays (16S rRNA, *bcsp31 and omp2* gene sequences) revealed poor diagnostic efficiency of 16S rRNA on bovine blood samples, while *bcsp31* was most sensitive and had similar sensitivity to *omp2* PCR (Mukherjee et al., 2007).

In the present study, the *Bcsp-31* (B4/B5) gene derived from polymorphic region for genus was employed to identify *Brucella*. Out of the 18 samples analyzed through conventional PCR, two samples were positive for *Brucella* genus. The sequences were aligned with reported sequences available in NCBI GenBank and revealed nearly 100% amino acid sequence-based identity with the *bcsp31* gene of *Brucella* reported from other parts of the world. Since inter-strain genomic variability was not observed, the occurrence of the disease would appear to be a result of the transportation of diseased animals. Gwida et al. (2011) revealed that bcsp31 kDa real-time PCR detected *Brucella* DNA in 84.8% (759/895) of the examined samples of camels in Dubai, UAE imported from infected herds of Sudan. Possible explanation of the difference in percentage of detection out of serological positive samples could be due to stage of the disease and higher sensitivity of real-time PCR compared to conventional PCR. In acute cases, molecular methods are useful techniques to detect organism at early stage of the disease, whereas in chronic cases, when organism is not present in the blood stream, serological tests are helpful.

Conclusion

The present study indicated the endemicity of brucellosis in camel especially in Faisalabad district of Punjab, Pakistan at a very low level similar to many research findings, along with its probable implication in human beings. The endemicity of the disease in Pakistan is of concern as livestock from one region is a potential source of infection for livestock in disease-free regions. This low prevalence allows the possibility of its control through test-and-slaughter strategy. However, detection of pathogen should be used for conformation of disease where the adoption of test-and-slaughter strategy may not be feasible. Moreover, the risk of spread of the disease due to uncontrolled movement of animals, poor hygiene and management conditions and free sale of infected animals in the markets cannot be overlooked. Deficiency of awareness of brucellosis with prevailing routine habit of consumption of raw milk and close contact with infected animals can function as mean of infection to human beings.

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บทคัดย่อ

ความชุกทางซีรัมวิทยา และการตรวจสอบระดับโมเลกุลของโรคบรูเซลโลซีสในอูฐ ในภูมิภาคของปากีสถาน

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อูฐมีบทบาทสำคัญในด้านความมั่นคงทางอาหารในประเทศกำลังพัฒนา โดยเฉพาะอย่างยิ่งในพื้นที่ที่แห้งแล้ง โรคบรูเซลโลซีสเป็นโรคที่มีความสำคัญ เนื่องจากเป็นโรคสัตว์สู่คน ซึ่งอูฐสามารถแพร่เชื้อสู่มนุษย์ผ่านการดื่มน้ำนม อย่างไรก็ตามในประเทศปากีสถาน การศึกษาโรคบรูเซลโลซีสในอูฐยังมีความจำกัด การศึกษาครั้งนี้ได้เก็บตัวอย่างซีรั่ม (n = 761) จากฝูงอูฐที่มีประวัติการแท้งจากสถานที่ต่าง ๆ ในจังหวัดปัญจาบ ประเทศปากีสถาน จากนั้นตรวจคัดกรองด้วยวิธี Rose Bengal Plate Test (RBPT) และตัวอย่างที่ให้ผลบวกจะนำมาทดสอบด้วยวิธี cELISA จากนั้นในตัวอย่างที่ให้ผลบวกต่อ RBPT และ cELISA ได้นำมาตรวจพิสูจน์หาเชื้อแบคทีเรียในระดับโมเลกุลด้วยวิธี PCR ผลการศึกษาพบว่า อูฐ 3.41% (n = 26, 95% CI 2.24-4.97) ให้ผลบวกต่อ RBPT ซึ่งความชุกของโรคบรูเซลโลซีส แตกต่างกันอย่างมีนัยสำคัญในสัตว์ ตามแหล่งภูมิศาสตร์ เพศ อายุ อายุการให้นม และสุขภาพสัตว์ ส่วนผลการศึกษาระดับโมเลกุลพบว่า มีเพียง 2 ตัวอย่าง ที่ตรวจพบเชื้อแบคทีเรียด้วยวิธี PCR และผลการศึกษารหัสพันธุกรรมของเชื้อ เปรียบเทียบกับรหัสพันธุกรรมที่รายงานในฐานข้อมูล GeneBank และพบว่ามีรหัสพันธุกรรมเหมือนกับยีน bcsp-31 (100%) ของเชื้อที่รายงานจากส่วนอื่นๆ ของโลก ผลการศึกษานี้ชี้ให้เห็นถึงผลกระทบทางด้านระบาดวิทยา เศรษฐกิจและการสาธารณสุขของโรคบรูเซลโลซีสในอูฐ ซึ่งเป็นประโยชน์ในการวินิจฉัยและควบคุมโรคที่มีประสิทธิภาพ

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