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Sentinel surveillance of selected veterinary and public health pathogens in camel population originating from Southern Punjab province, Pakistan

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ABSTRACT

An extended range of host susceptibility including camel has been evidenced for some of the important veterinary and public health pathogens, such as brucellosis, peste des petits ruminants (PPR) and bluetongue (BT). However, in disease endemic settings across many parts of the globe, most of the disease control interventions accounts for small and large ruminants, whereas unusual hosts and/or natural reservoirs, such as camels, remain neglected for disease control measures including routine vaccination. Such a policy drawback not only plays an important role in disease epizootiology particularly in settings where disease is endemic, but also serves an obstacle in disease control and subsequent eradication in future. With this background, using pre-validated ELISA and molecular assays [multiplex PCR, reverse transcriptase (RT)-PCR and real-time (rt)-PCR], we conducted a large-scale pathogen- and antibody-based surveillance for brucellosis, peste des petits ruminants and bluetongue in camel population (n = 992) originating from a wide geographical region in southern part of the Punjab province, Pakistan. Varying in each of the selected districts, the seroprevalence was found to be maximum for bluetongue [n = 697 (70.26%, 95% CI: 67.29–73.07)], followed by PPR [n = 193 (19.46%, 95% CI: 17.07–22.09)] and brucellosis [n = 66 (6.65%, 95% CI: 5.22–8.43)]. Odds of seroprevalence were more significantly associated with pregnancy status (non-pregnant, OR = 2.23, 95% CI: 1.86–5.63, $p<0.01$), farming system (mixed-animal, OR = 2.59, 95% CI: 1.56–4.29, $p<0.01$), breed (Desi, OR = 1.97, 95% CI: 1.28–4.03, $p<0.01$) and farmer education (illiterate, OR = 3.17, 95% CI: 1.45–6.93, $p<0.01$) for BTV, body condition (normal, OR = 3.54, 95% CI: 1.92–6.54, $p<0.01$) and breed (Desi, OR = 2.19, 95% CI: 1.09–4.40, $p<0.01$) for brucellosis, and feeding system for PPR (grazing, OR = 2.75, 95% CI: 1.79–4.22, $p<0.01$). Among the total herds included (n = 74), genome corresponding to BT virus (BTV) and brucellosis was detected in 14 (18.92%, 95 CI: 11.09–30.04) and 19 herds (25.68%, 95% CI: 16.54–37.38), respectively. None of the herds was detected with genome of PPR virus (PPRV). Among the positive herds, serotype 1, 8 and 11 were detected for BTV while all the herds were exclusively positive to *B. abortus*. Taken together, the study highlights the role of potential disease reservoirs in the persistence and transmission of selected diseases in their susceptible hosts and, therefore, urges necessary interventions (e.g., inclusion of camels for vaccine etc.) for the control of diseases from their endemic setting worldwide.

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1. Introduction

Camel (*Camelus dromedarius*) has an ability to survive hot and arid environmental conditions due to its unique physiological system. Therefore, it imparts an important role in the routine livelihood of millions of people inhabiting desert areas across Asia and Africa (Schwartz 1992; Gwida et al., 2012). Together its strength and docility makes it an ideal animal that could be used for multiple purposes including agriculture-related activities, such as ploughing the fields, crushing of sugarcanes, grinding seeds and grains, and transportation etc. (Iqbal, 1999; Khan et al., 2003).

Earlier it was believed that camel is resistant to many infectious diseases of veterinary and public health significance; however, it is now evidenced otherwise and several bacterial (e.g., *Brucella, E. coli* etc.) and viral infections (e.g., bluetongue virus [BTV], Middle-East respiratory syndrome coronavirus [MERS CoV] etc.) have been reported. For instance, beside isolation of *Enterococcus* spp. and *Salmonella* spp., Al-Ruwaili et al. (M.A., 2012) reported seroprevalence for *E. coli, Brucella abortus* and group A rotavirus. Fouda and Al-Mujalii (2007) reported clinical infection caused by *Proteus* spp., *Staphylococcus aureus,* and *Clostridium perfringens.* Resulting either from rearing in close vicinity to small and large ruminants such as cattle, sheep and goat or an experimental infection, clinical susceptibility of camels to *B. abortus* and *B. melitensis* has also been reported previously (Damir et al., 1989; Hamdy and Amin, 2002). Similarly, for viral infections in camels, seroprevalence (Abraham et al., 2005; Swai et al., 2011; Woma et al., 2015) and sequence-based phylogeny has been revealed for peste des petits ruminants (PPR) (Omani et al., 2019). Seroprevalence, either upon natural exposure (Chandel et al., B. 2003) or experimental infection (Batten et al., 2011), also indicate its susceptibility to BTV.

For an agriculture-based economy worldwide, such as Pakistan, rearing small and large ruminants is an integral part of such a production system. However, despite the use of necessary preventive and disease control measures, repeated occurrence of infectious diseases in small and large ruminants is not uncommon resulting in subsequent economic losses in terms of morbidity, mortality and trade embargo (e.g., in case of brucellosis and PPR). While an extended range of host susceptibility including camel has been evidenced for some pathogens, such as brucellosis (Abbas and Agab, 2002; Gwida et al., 2012), PPR (Abraham et al., 2005; Swai et al., 2011; Woma et al., 2015) and BT (Chandel et al., 2003), most of the disease control interventions accounts for small and large ruminants whereas unusual hosts and/or natural reservoirs, such as camels, remain neglected for any of such disease control initiatives, including routine vaccination. Such a policy drawback not only plays an important role in disease epizootiology, particularly in settings where these diseases are endemic, but also serves an obstacle in its control and subsequent eradication, such as proposed by OIE/FAO for PPR. This is important to consider because, with an overall population of 1.2 million heads, Pakistan ranks 8th among major camel raising countries around the globe and does produce 908,000 tons of milk and 50,603 tons of meat per annum for human consumption (Economic Survey of Pakistan 2018–19). With this background, we conducted a large-scale antigen (pathogen)- and antibody-based surveillance for three of the most important endemic infectious diseases (brucellosis, PPR and BT) in camels representing a wide geographical region in the Punjab province of Pakistan. Providing a prevalence status for each of the infectious diseases, the study outcomes are expected to ascertain necessary interventions for disease management and control particularly in a setting where there is frequent contact among ruminants and camels.

2. Materials and methods

2.1. Study area

Southern Punjab accounts for 52% of agro-ecological landscape of the Punjab province where there inhabits 32% of province population (https://nation.com.pk/06-May-2012/south-punjab-larger-in-size-less-in-population). Socioeconomic activities in this particular region are mostly centered for agriculture and livestock production for human livelihood. The region has an arid climate with an extreme weather conditions such as hot summer and cold winter where the recorded average temperature and humidity is 25.6 ºC and 48.0% (https://weather-and-climate.com/average-monthly-Humidity-perc,Multan,Pakistan), respectively. Within this region, there exists a dense population of camels in a desert area called “Cholistan”. The camel rearing or production system is largely dominated by the sedentary farming system followed by nomadic and/or transhumant system. Sedentary system involves camel rearing in a designated land along with small and large ruminants, while nomadic/transhumant system is characterized by diversification of herds with small ruminant, seasonal migration for feed and water, and loaning/sharing of herd or animals within a herd. Despite importance of camels in meeting the routine livelihood of individual/s involved in each type of the rearing systems, there is an absolute paucity of proper farming and husbandry practices exclusive for camels and they are usually kept along with domestic ruminants including sheep, goat, cattle and buffalo. Such a mixed livestock production practice most likely supports an increased incidence of disease/s which has potential to transmit across the species. Though, in the recent past, L&DD Punjab has conducted a mass-scale campaign for camel population enumeration, farmer's awareness on camel diseases and their management, deworming, trainings to the farmers and veterinarian in the area (http://livestockpunjab.gov.pk/LiveStockAdmin/uploads/item_files/camel_project_njbnu.pdf); howeve other than hemoparasites and/or fecal parasites examination, there lacks a continuously updated system for vaccination, disease monitoring and surveillance in camels across the Punjab province.

2.2. Sample collection

Of the 11 districts of Southern Punjab, we included seven districts named Bahawalpur, Dera Ghazi (DG) Khan, Multan, Muzaffargarh,

Table 1
District-wise percent seroconversion against Brucellosis, Peste des Petits ruminants and bluetongue in individual small and large ruminant in Punjab province Pakistan .

Districts	Samples	Brucella			PPR			BTV		
		+ve	%	95% CI	+ve	%	95% CI	+ve	%	95% CI
Bahawalpur	30	2	6.67%	1.16–23.51	8	26.67%	12.98–46.18	21	70%	50.44–84.59
DG Khan	207	16	7.73%	4.63–12.47	45	21.74%	16.45–28.11	169	81.64%	75.54–86.53
Multan	100	9	9.00%	4.46–16.83	8	8.00%	3.77–15.61	68	68.00%	57.82–76.78
Muzaffar Garh	17	0	0.00	0.00	0	0.00%	0.00	17	100.00%	77.08–100
Rajan Pur	152	20	13.16	8.42–19.83	8	5.26%	2.47–10.45	34	22.37%	16.19–29.98
RY Khan	254	8	3.15	1.47–6.34	89	35.04%	29.25–41.29	169	66.54%	60.33–72.24
Taunsa	232	11	4.74	2.51–8.55	35	15.09%	10.87–20.50	219	94.40%	90.39–96.86
Total	992	66	6.65%	5.22–8.43	193	19.46%	17.07–22.09	697	70.26%	67.29–73.07

Rahim Yar (RY) Khan, Rajan Pur and Taunsa, to assess the pathogen and antibody-based surveillance of selected infectious diseases (brucellosis, PPR and BT) in the camel population. These districts represent typical agro-climatic conditions of the region where, besides the presence of a large number of livestock population including small and large ruminants, dense population of camels exist.

Following necessary approvals from ethical research committee of the University of Veterinary and Animal Sciences vide letter no. Dr/236 dated May 16, 2017 and farmer's consent, approximately 3 ml blood (n = 992) were collected in gel-clot activator tubes (Vacuette®, VWR, USA), as aseptically as possible using appropriate personal protective equipment. Corresponding to respective herds (Table 1), the collected samples represented RY Khan (n = 254), Taunsa (n = 232), DG Khan (n = 207), Rajan Pur (n = 152), Multan (n = 100), Bahawalpur (n = 30) and Muzaffargarh (n = 17). Each blood sample was centrifuged at 3000 xg for 10 min. Serum was separated in a pre-labelled 2.0 ml cryovial and stored at −20 °C until further used for pathogen- and antibody-based assay. Alongside sample collection, detailed information regarding several categorical variables including breed (Desi, other), gender (male, female), age group (<14.5, 14.5–28, 28–41.5 months), body condition (normal, weak), pregnancy status, feeding system (intensive, extensive, mixed), farming system (mixed farm, camels only) and farmer's education (literate, illiterate) was recorded. An intensive feeding system correspond to camels grazing in natural environment, an extensive feeding system correspond to manger feeding whereas the mixed system is comprised of both manger feeding and grazing of animals in the natural environment. Mixed farming system included camels reared with other pastoralists or agro-pastoralists.

2.3. Seroprevalence for brucellosis, peste des petits ruminants and bluetongue

Commercially available ELISA kits were used to determine the seroprevalence for brucellosis (ID Screen® Brucellosis Serum Indirect Multi-Species, Grables, France), PPR (anti-PPRV nucleoprotein based cELISA kit supplied by CIRAD-EMVT, Montpellier, France), and bluetongue (ID Screen® Bluetongue Competition, Grables, France) as per manufacturer's instructions. Each of the kit had potential to detect immunoglobulin (IgG) to a relatively conserved epitope, such as lipopolysaccharide for *Brucella* spp., nucleoprotein for PPRV, and VP7 for bluetongue virus. Antibodies to *Brucella* spp. were detected employing direct antigen and antibody interaction (iELISA) while, both for PPR and bluetongue, there was a competition between subject antibody and kit-provided anti-antibodies (competitive ELISA). For a sera positive or negative to one of the selected pathogens, the development of color intensity varied accordingly and measured in terms of optical density (OD) (iMark™ Microplate Absorbance Reader, BioRad, USA) at 450 nm for both brucellosis and BT, while it was 492 nm for PPR. For brucellosis, sample to positive control percentage (S/P%) was calculated ($S/P\% = OD_{sample}–OD_{NC}/OD_{PC}–OD_{NC} \times 100$). Sera that showed S/P% less than or equal to 110 were considered negative, those that showed 110% < SP < 120% were considered doubtful while those that showed S/P% greater than 120 were considered positive. For PPR, threshold value (cut-off value) of 50% was determined for each of the sera ($PI = 100–[(OD_{sample}/OD_{control}) \times 100]$. Sera that showed percentage inhibition (PI) <50% were considered negative, while sera with >50% PI were considered positive. Similarly, for BT, the signal-to-noise ratio was calculated ($S/N\% = OD_{sample}/OD_{NC} \times 100$). Sera with S/N % ≥ 40% were considered negative, while those that showed S/N % < 40% were considered positive.

2.4. Genomic identification and serotyping of brucellosis, peste des petits ruminants and bluetongue

Irrespective of the sera outcome, i.e., whether or not seropositive to study-included pathogen, a pool of 5–6 sera was made representing each herd. Each of the pooled sera was processed separately for extraction of genomic DNA (FavorPrep™ Genomic DNA Extraction Kit, Favorgen®, BioTech Corp., Austria) and RNA (QIAamp Viral RNA Extraction Mini Kit, Qiagen, Valencia, CA) as per manufacturer's instructions. Quality ($A_{260/280}$ and $A_{260/230}$) and quantity of genome (ng/μl) was estimated (NanoDrop, USA) and processed further for genome amplification using pathogen-specific genetic markers.

For species-specific identification of *Brucella*, the genomic DNA samples were first screened with genus-specific forward (5′-TGG CTC GGT TGC CAATAT CAA-3′) and reverse (5′-CGC GCT TGC CTT TCA GGT CTG-3′) primers targeting bcsp31 gene. Samples that showed a positive amplification (223 bp) were subjected further for a multiplex PCR for species identification. A set of primers targeting IS711 gene for each of the *Brucella* species was used accordingly with a varying product size. These included forward (5′-GAC GAA CGG AAT TTT TCC AAT CCC-3′) and reverse primer (5′-TGC CGA TCA CTT AAG GGC CTT CAT-3′) for *B. abortus* yielding a product of 498 bp, forward (5′-AAA TCG CGT CCT TGC TGG TCT GA-3′) and reverse primer (5′-TGC CGA TCA CTT AAG GGC CTT CAT-3′) for *B. melitensis* yielding a product of 731 bp, forward (5′-CGG GTT CTG GCA CCA TCG-3′) and reverse primer (5′-TGC CGA TCA CTT AAG GGC CTT CAT-3′) for *B. ovis* yielding a product of 976 bp, and forward (5′-GCG CGG TTT TCT GAA GGT TCA GG-3′) and reverse primer (5′-TGC CGA TCA CTT AAG GGC CTT CAT-3′) for *B. suis* yielding a product of 285 bp.

For PPRV, one-step reverse-transcriptase polymerase chain reaction (RT-PCR) was employed to amplify genomic RNA corresponding to nucleoprotein gene (*NP*) of peste-des-petits-ruminants virus (PPRV) following a previously published protocol (Couacy-Hymann et al., 2002). Along with positive (vaccine strain Nigeria 75/1) and negative controls (normal saline), the assay used forward (5′-TCTCGGAAATCG CCTCACAGACTG-3′) and reverse (5′-CCTCCTCCTGGTCCTCCAGAA TCT-3′) primers giving a product of 351 bp on agarose gel (2.0%). For identification of prevailing serotype of bluetongue virus, the extracted genomic RNA was subjected to rt-PCR-based genotyping through a commercially available kit (LSI VetMAX European BTV Typing Real Time PCR kit (Thermo-Fisher Scientific LSI, Lissieu, France). The kit had potential to selectively detect eight different serotypes (1, 2, 4, 6, 8, 9, 11 and 16) when performed in a separate reaction along with positive, negative and internal controls. As per manufacturer's instruction, separate reaction for each of the genotypes were set-up, followed by thermal cycling in a CFX96™ Real-time PCR Detection System (Bio-Rad, California, USA) and recording of Ct-values. For each of the serotype analyzed, samples that showed Ct < 40 were taken as positive for genome corresponding to specific BTV serotype.

2.5. Statistical analysis

The data were managed and analyzed within R software version 3.4.4 (lme4 package). The prevalence of individual infection and co-infection was graphed using stacked bars. For each type of infection, the association between putative risk factors and infection was determined through chi-square test. In precision of accuracy of assumption of independence of sample in chi-square test, we also used Fisher–Exact test as alternate statistical approach. Correlation coefficient among variables was measured by Cramer's V statistic and the value were ≤ 0.2. For univariate analysis, a p-value equal to 0.20 was taken as a criterion for inclusion/exclusion of any risk factor for multivariate analysis. Logistic regression analysis was performed to calculate odds ratio and 95% CI. We did not consider co-infections for univariate and multivariate because numbers of animals in a particular risk factor for a study-included disease matrix were small resulting in abnormal ORs and 95% CI. A map showing spatial distribution of the infections was created with QGIS version 3.8.0.

3. Results

The study included a total of 992 sera representing seven selected districts of Southern Punjab, Pakistan for surveillance for brucellosis, PPRV and BTV (Fig. 1). Overall, a varying rate of prevalence was observed for each of the study pathogen in the tested animals. Seroprevalence was found to be 6.65% (95% CI: 5.22–8.43) for brucellosis, 19.46% (95% CI: 17.07–22.09) for PPR, while it was found to be 70.26% (95% CI: 67.29–73.07) for BT. Rate of seroprevalence of brucellosis was more in camels originating from district Rajan Pur (13.16%, 95% CI: 8.42–19.83), while it was least in camels from district RY Khan (3.12%, 95% CI: 1.47–6.34). The proportion of seropositivity for PPR was more in camels originating from district RY Khan (35.04%, 95% CI: 29.25–41.29), whereas it was the least in camels from Rajan Pur district (5.26%, 95% CI: 2.47–10.45). None of the camels was seropositive from district Muzaffar Garh for both of brucellosis and PPR. Similarly, for bluetongue, all the tested animals were seropositive in Muzaffar Garh (100%, 95% CI: 77.08–100.0), while it was least in camels from district Rajan Pur (22.37%, 95% CI: 16.19–29.98) (Table 1). For seroprevalence to more than one pathogen, a maximum rate of seropositivity was observed for bluetongue and PPR infection (n = 140, 14%, 95% CI: 12.08–16.42), followed by bluetongue and brucellosis (n = 34, 4%, CI: 2.47–4.75), and PPR and brucellosis (n = 10, 1.0%, CI: 0.5–1.85). Percent positivity indicating a cumulative exposure to brucellosis, PPR and bluetongue infection was observed exclusively in 1.0% of the studied camel population (n = 10, CI: 0.5–1.85) (Fig. 2). A district-wise distribution of percent positivity of each of the study pathogen either alone or cumulatively is indicated in the Fig. 1.

The tested sera representing 74 herds of camels were distributed from the selected districts as follows: district Taunsa (n = 17) RY Khan (n = 16), DG Khan (n = 15), Rajanpur (n = 11), Multan (n = 10), Bahawalpur (n = 3) and Muzaffar Garh (n = 2). Most herds were seropositive to bluetongue (n = 71, 95.95%, CI: 87.82–98.94), followed by PPR (n = 54, 72.97%, 95 CI: 61.19–82.33) and brucellosis (n = 31, 41.89%, 95% CI: 31.32–53.26). Herd-based prevalence for bluetongue was high (100%) in each of the district Bahawalpur (n = 3), DG Khan (n = 15), Multan (n = 10), Muzaffar Garh (n = 2), RY Khan (n = 16) and Taunsa (n = 17), while it was least (72.73%) in district Rajan Pur (n = 8). Herd-based prevalence for PPRV infection was high (100%) in each of the district Bahawalpur (n = 3), and RY Khan (n = 16), whereas it was least (36.35%) in district Rajan Pur (n = 4). Similarly, herd-based prevalence for brucellosis was high (63.64%) in district Rajan Pur (n = 7), while it was least (33.33%) in district Bahawalpur (n = 1). None of the herds was seropositive from district Muzaffar Garh for both of PPR and brucellosis (Table 2).

Univariate analysis was used to assess an association between different categorical variables and occurrence of seropositivity to each of the studied pathogens in camels. Excluding a few of the variables for each of the pathogen, all showed an initial association at $p < 0.20$ with the exposure to specific pathogen indicated by the seroprevalence. These few variables included feeding system (p = 0.888) and farmer's education (p = 0.585) for brucellosis, and gender (p = 0.399), type of farming (p = 0.521) and age group (p = 0.503) for PPR. Notably, all variables showed significant association with the occurrence of bluetongue infection (Table 3). However, when further analyzed through

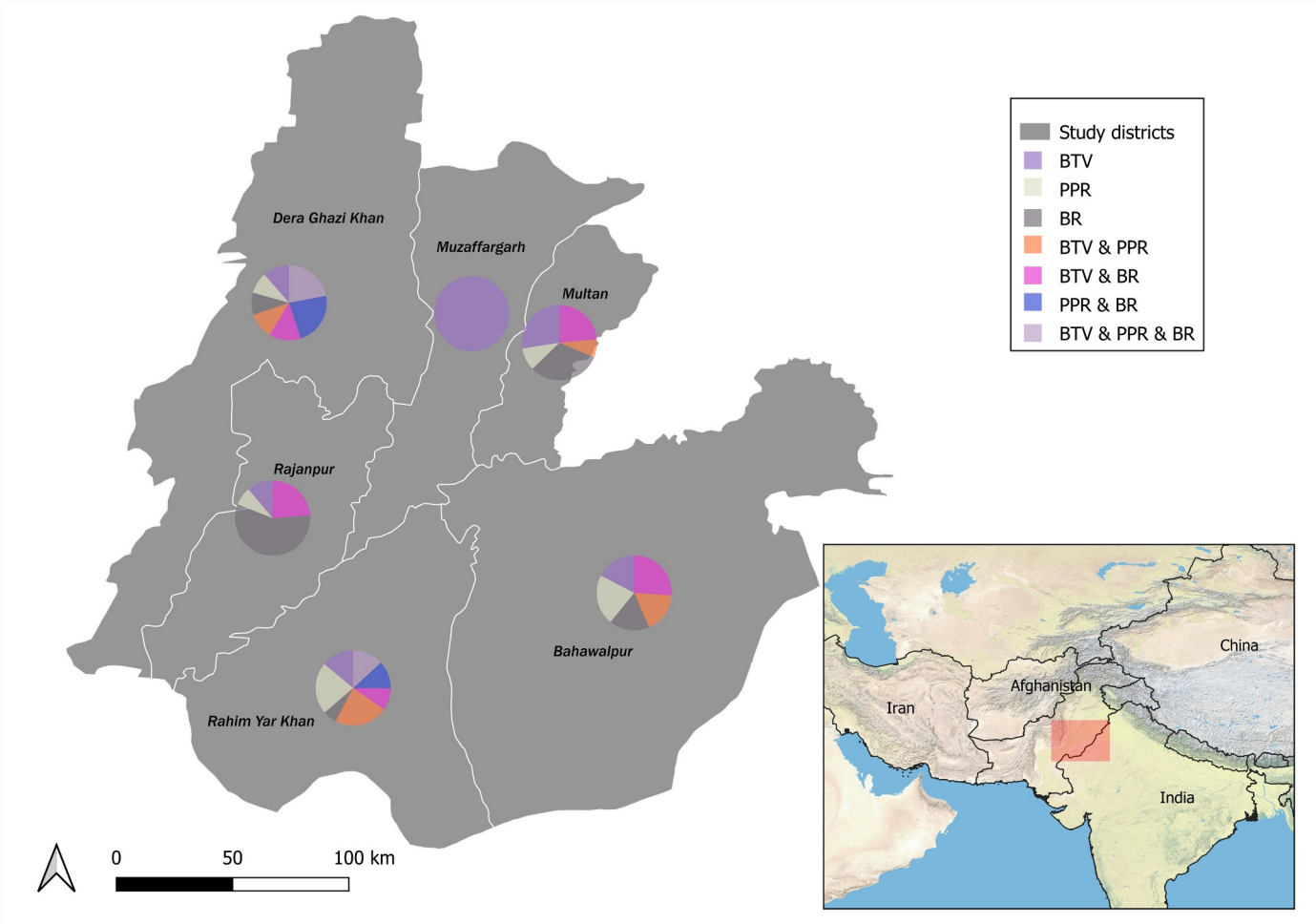

Fig. 1. Geographical seropositivity of *Brucella* (BR), PPRV and BTV in camel population of selected districts of Southern Punjab, Pakistan.

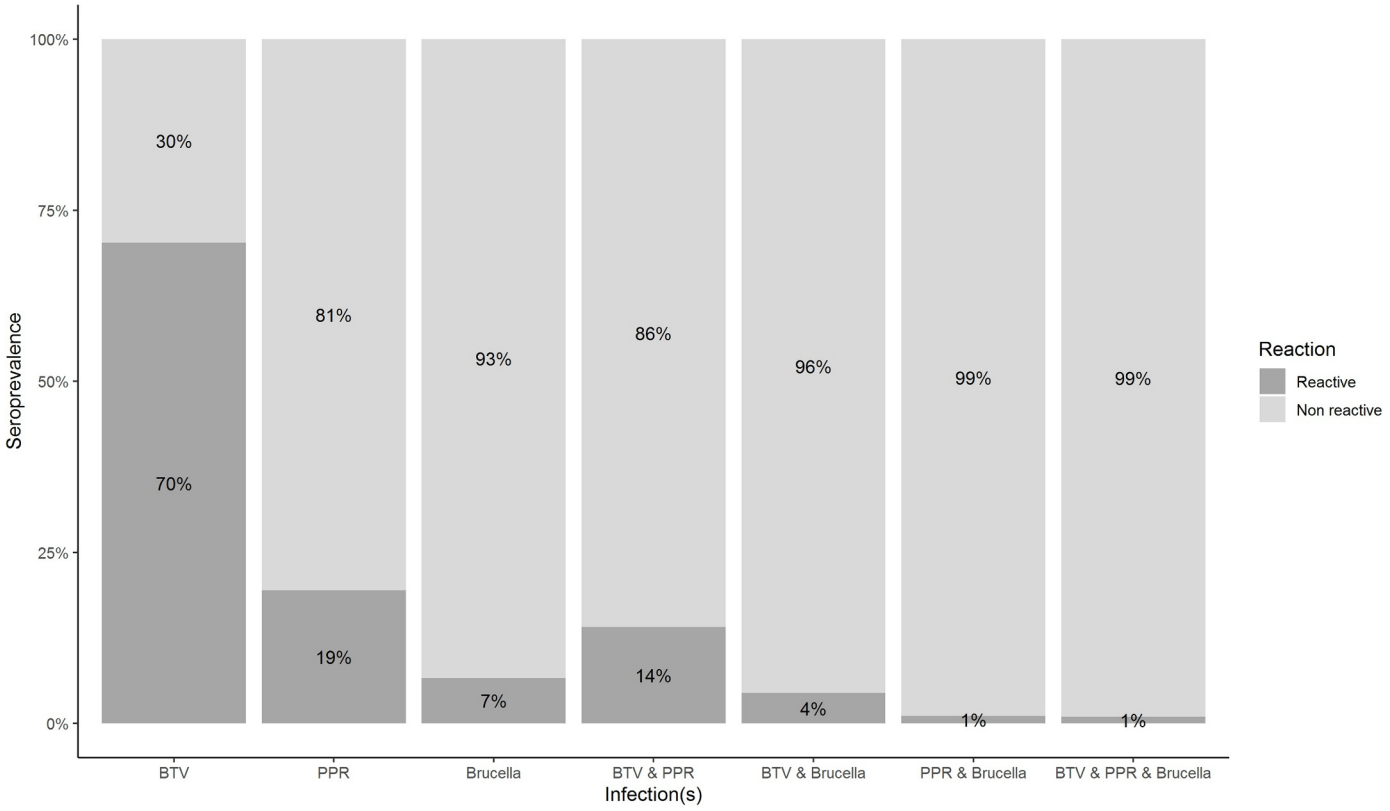

Fig. 2. Individual and coinfection-based reactivity for presence of antibodies against Brucella, PPRV and BTV in camel population.

multivariate logistic regression analysis, pre-determined initial association became degenerative and only a few of the variables had a significant association with the occurrence of seropositivity in camels. The odds of occurrence of seropositivity in brucellosis was more significant ($p < 0.01$) in animals with poor body condition (OR = 3.54, 95% CI: 1.92–6.54) and of Desi breed (OR = 2.19, 95% CI: 1.09–4.40). For PPR, the odds of occurrence of seropositivity were more significant ($p < 0.01$) in animals that had mixed feeding system (OR = 2.75, 95% CI: 1.79–4.22). Similarly, the odds of BT occurrence were more significant ($p < 0.01$) in non-pregnant animals (OR = 2.23, 95% CI: 1.86–5.63), intensive farming system (OR = 2.59, 95% CI: 1.56–4.29), Desi breed (OR = 1.97, 95% CI: 1.28–4.03) and depending on farmer's education (OR = 3.17, 95% CI: 1.45–6.93) (Table 4).

While genome-based identification and further subtyping of each of the study pathogen is concerned, only genome corresponding to *Brucella* spp. and BTV was identified. All the processed samples were found negative to genome corresponding to nucleoprotein gene of PPRV. Out of 74 camel herds, genome conforming to *Brucella* spp. and BT was detected among 19 (25.68%, 95% CI: 16.54–37.38) and 14 camel herds (18.92%, 95% CI: 11.09–30.04), respectively. Further multiplex-PCR based species identification revealed all of the *Brucella* spp. positive herds exclusively to be *Brucella abortus*. A maximum number of camel herds were positive from district Taunsa and Rajan pur each ($n = 6$, 8.11%, 95% CI: 3.34–17.43), followed by RY Khan ($n = 4$, 5.41%, 95% CI: 1.75–13.99) and Multan ($n = 3$, 4.05%, 95% CI: 1.05–12.18). Similarly, for BTV, a higher number of camel herds representing district Multan were found antigen positive ($n = 8$, 10.81%, 95% CI: 5.11–20.72), followed by Taunsa ($n = 3$, 4.05%, 95% CI: 1.05–12.18), Muzaffar Garh ($n = 2$, 2.70%, 95% CI: 0.47–10.30) and DG Khan ($n = 1$, 1.35%, 95% CI: 0.07–8.32). BTV serotype distribution was found to be district-specific, where serotypes 8 and 11 were detected from camel herds in district Multan, while serotypes 1 and 8 were exclusively detected from camel herds originating from district DG Khan, Muzaffar Garh, and Taunsa, respectively.

4. Discussion

Continuous disease epidemiology and surveillance is considered

Table 2
Herd-wise seroconversion and antigen detection in small and large ruminants across select districts of Punjab province Pakistan.

Districts	Total Herds	#Brucella			*PPR			BTV		
		+ve	%	95% CI	+ve	%	95% CI	+ve	%	95% CI
Bahawalpur	3	1	33.33%	1.76–87.47	3	100.0%	31.00–100.0	3	100.0%	31.00–100.0
DG Khan	15	7	46.67%	22.28–72.58	12	80.00%	51.37–94.69	15	100.0%	74.65–100.0
Multan	10	4	40.00%	13.69–72.63	6	60.00%	27.37–86.31	10	100.0%	65.55–100.0
Muzaffar Garh	2	0	00.00%	0.00	0	00.00%	00.00	2	100.0%	19.79–100.00
Rajan Pur	11	7	63.64%	31.62–87.64	4	36.36%	12.36–68.38	8	72.73%	39.32–92.67
RY Khan	16	6	37.50%	16.28–64.13	16	100.%	75.93–100.0	16	100.%	75.93–100.0
Taunsa	17	6	35.29-	15.26–61.38	13	76.47%	49.76–92.18	17	100.0%	77.08–100.0
Total	74	31	41.89%	31.32–53.26	54	72.97%	61.19–82.33	71	95.95%	87.82–98.95

Brucella abortus was detected in positive herds across the select districts.
* None of the herd was detected for genome corresponding to small ruminant morbillivirus.

Table 3
Univariate analysis of seropositive camels against Brucellosis, PPR and BT and their potential association to categorical variables.

Categorical variables	Total N = 992 (%)	Brucellosis Reactive N = 66 (%)	p-value	PPR Reactive N = 193 (%)	p-value	BT Reactive N = 697 (%)	p-value
Gender							
Female	635 (64.0)	55 (83.3)	<0.01	118 (61.1)	0.399	472 (67.7)	<0.01
Male	357 (36.0)	11 (16.7)		75 (38.9)		225 (32.3)	
Body condition							
Normal	856 (86.3)	46 (69.7)	<0.01	176 (91.2)	0.037	633 (90.8)	<0.01
Weak	136 (13.7)	20 (30.3)		17 (8.8)		64 (9.2)	
Pregnancy status							
Non-pregnant	853 (86.0)	52 (78.8)	0.119	176 (91.2)	0.027	576 (82.6)	<0.01
Pregnant	139 (14.0)	14 (21.2)		17 (8.8)		121 (17.4)	
Feeding system							
Intensive	546 (55.0)	37 (56.1)	0.888	76 (39.4)	<0.01	375 (53.8)	0.019
Extensive	249 (25.1)	15 (22.7)		48 (24.9)		192 (27.5)	
Mixed	197 (19.9)	14 (21.2)		69 (35.8)		130 (18.7)	
Type of farming							
Mixed farm	825 (83.2)	65 (98.5)	<0.01	164 (85.0)	0.521	553 (79.3)	<0.01
Camels only	167 (16.8)	1 (1.5)		29 (15.0)		144 (20.7)	
Breed							
Desi	778 (78.4)	44 (66.7)	<0.01	167 (86.5)	<0.01	525 (75.3)	<0.01
Other	214 (21.6)	22 (33.3)		26 (13.5)		172 (24.7)	
Age group (months)							
<14.5	966 (97.4)	61 (92.4)	0.019	187 (96.9)	0.503	674 (96.7)	0.082
14.5–28	23 (2.3)	4 (6.1)		6 (3.1)		21 (3.0)	
28–41.5	3 (0.3)	1 (1.5)		0 (0.0)		2 (0.3)	
Farmer's Education							
Non-literate	923 (93.0)	63 (95.5)	0.585	188 (97.4)	0.012	636 (91.2)	<0.01
Literate	69 (7.0)	3 (4.5)		5 (2.6)		61 (8.8)	

Table 4
Multivariate analysis of potentially associated categorical variables against Brucellosis, PPR and BT in camels.

Variable	Brucellosis Odds Ratio (95% CI)	p-value	PPR Odds Ratio (95% CI)	p-value	BT Odds Ratio (95% CI)	p-value
Body condition						
Normal *Weak*	3.54 (1.92–6.54)	<0.01	....		....	
Pregnancy status						
Non-pregnant Pregnant	....		....		2.23 (1.86–5.63)	<0.01
Feeding system						
Intensive Extensive Mixed	....		2.75 (1.79–4.22)	<0.01	....	
Farming system						
Mixed farm Camels only	....		....		2.59 (1.56–4.29)	<0.01
Breed						
Desi Other	2.19 (1.09–4.40)	<0.01	....		1.97 (1.28–4.03)	<0.01
Farmer's Education						
Non-literate *Literate*	....		....		3.17 (1.45–6.93)	<0.01

Note: The significant variable is italic.

important for control and eradication of disease/s across disease endemic settings worldwide (Klepac et al., 2015). Therefore, for a number of infectious diseases, time-to-time antigen and antibody detection-based surveillance has previously been conducted in small and large ruminants in Pakistan. Nevertheless, except for a few studies on brucellosis in camels (Shahzad et al., 2017; Baloch et al., 2017), there is an absolute paucity of data on surveillance of PPR and BT, and subsequent necessary interventions in terms of evidence-based decision making to ultimately improve veterinary and public health, the environment and the society. An integration of animal health surveillance into an economic framework has indicated no or a very limited economic value; however, when combined with decisions on interventions, surveillance contribute to disease mitigation and therefore justify the resources used (Howe et al., 2013). This can fairly be evidenced for Foot and Mouth disease in Pakistan where disease control and eradication strategies are taken as top-priority by national and international organizations (FAO/OIE). With this background understanding, we conducted pathogen- and antibody-based surveillance for selected veterinary and public health pathogens in the camel population of Southern Punjab, Pakistan. An indirect antigen-antibody interaction (iELISA) was employed for the detection of anti-*Brucella* antibodies, while competitive ELISAs were used for the detection of antibodies for PPR and bluetongue. The used assays are highly efficient, sensitive and specific for each of the selected pathogens and are considered reliable for sero-surveillance of brucellosis, BT and PPR (Paweska et al., 2002: Biteau-Coroller et al., 2006; Bodjo et al., 2018).

Overall, a high seroprevalence (70.26%) to BT followed by PPR (19.46%) and brucellosis (6.65%) was found in the targeted camel population of Southern Punjab, Pakistan. Such a high level of seroprevalence of these pathogens is not surprising because of the reported endemicity of the studied pathogens in livestock populations of the Punjab province (Khan et al., 2008; Abubakar et al., 2010; Aziz-ul-Rahman et al., 2016, 2017; Shabbir et al., 2018; T. Sohail et al., 2019). As can be evidenced from the annual reports of Livestock and Dairy Development department of the Punjab province (http://www.livestockpunjab.gov.pk/dashboard/lndd_punjab), a frequent occurrence of clinical cases of PPR and brucellosis in small and large ruminants are not uncommon, while for bluetongue, it is conceived to be sub-clinical. Since the camels are not included for any vaccination program for prevalent infectious pathogens in the country, particularly

those included in this study, the serum-based prevalence is truly an indication of natural exposure, either from the environment or via horizontal transmission while sharing vicinity or close proximity for housing and pasture with other livestock.

Varying from 22.37–100% among selected districts, an overall seroprevalence of 70.26% of BT in camel population indicates its high prevalence in the Punjab province. A varying prevalence of BT in camels has also been evidenced from neighboring countries, as well as other parts of the world, such as 67.8% in Iran (Mozaffari et al., 2013), 41.8% and 42% in Morocco (Touil et al., 2012; Drif et al., 2018), 25.7% in Saudi Arabia (Yousef, Al-Eesa and Al-Blowi, 2012), 21% in Algeria (Madani et al., 2011) and 19.3% in India (Chandel et al., 2003). Taking camels a potential reservoir of BTV, particularly in case of subclinical infection, either the movement or rearing of camels in mixed farming system could be speculated as one of the potential sources of infection to other susceptible hosts. This is important because recent studies have indicated a role of camelids as a disease reservoir that may lead to dissemination and persistence of BTV into the environment and, therefore, can result in an increased risk of infection in susceptible ruminants (Garcıa-Bocanegra et al., 2011; Batten et al., 2011). Though disease is believed to be sub-clinical in Pakistan, an increased prevalence in camel population in this study and those reported in the recent years in small and large ruminants in Punjab province (T. Sohail et al., 2019), and small ruminants in Baluchistan province (Sohail et al., 2018) and Khyber Pakhtun Khwan province (Malik et al., 2018) suggest otherwise and, therefore, urge to strengthen clinical expertise of veterinarians to a level enough to discriminate BT from other closely related clinical conditions, such as PPR and foot and mouth disease, and develop appropriate laboratory-based differential diagnostics. As far as genome-based serotyping of prevalent BTV is concerned in camel population, multiple serotypes (1, 8 and 11) were identified. The current observation agrees with most recent studies in the country where, dominated by serotype 8, a co-circulation of serotypes 2, 4, 6, 9, 11 and 16 in small and large ruminants originating from a wide geographical area has been evidenced (Sohail et al., 2018; Sohail et al., 2019; Malik et al., 2019). Hence, while living in close vicinity to small and large ruminants, an identification of serotypes 8 and 11 in camels is not unusual other than detection of serotype 1. Such an evidence argues for host-dependent potential of each of the BTV serotypes simply because, compared to other serotypes such as 4, 6, 8, 11, 14 and 16, a previous study observed an increased incidence of serotype 1 in camels (Drif et al., 2018). Though camels is generally conceived to be a non-susceptible host for BTV in the past, experimental investigations have suggested otherwise, where, particularly for serotype 1 and 8, a low rate of replication or infection along with development of neutralizing antibodies have been evidenced and therefore may serve as potential source of infection for susceptible hosts living in close proximity (Zanolari et al., 2010; Batten et al., 2011; Schulz et al., 2012). Thus, routine BT surveillance in camels should be conducted in disease endemic region/s in order to elucidate disease epidemiology and future interventions for its control.

District-wise, a varying rate (5.26% to 35.04%) of prevalence of antibodies for PPR was also evidenced in camel population. This is not unexpected because PPR is endemic in the country and viral genome and antibody-based prevalence has previously been reported in wild animals (Ibex), semi-domestic yak (Abubakar et al., 2019) and small ruminants (Khan et al., 2008; Zahur et al., 2008; Zahur et al., 2011; Abubakar et al., 2011). The virus generally causes infection in small ruminants worldwide (Al-Majali et al., 2008; Megersa et al., 2011; Intisar et al., 2017); however, it also has the propensity to cause infection in wild ungulates (Aziz-ul-Rahman et al., 2018, Rahman, 2019) and other unusual hosts such as buffalo, cattle (Balamurugan et al., 2014) and camels (Omani et al., 2019). Indeed, there exists a controversy regarding clinical infection of PPRV in camels (Ul-Rahman et al., 2020). A few studies have reported an occurrence of natural clinical infection with a mortality ranging from 60% − 90% (Khalafalla et al., 2010: Saeed et al., 2015), whereas another study claimed an absolute lack of clinical infection upon experimental exposure (Fakri et al., 2019). We observed a higher seroprevalence (19.46%) in camels as compared to the ones reported from Ethiopia (3.0% and 3.36%), Nigeria (7.9%) and Egypt (14.0%) (Roger et al., 2001; Haroun et al., 2002; Abraham et al., 2005; Woma et al., 2015). Such variations in seroprevalence may partly be attributed to differences in management system, health care practices, sample size, study periods, climate, socio-ecological factors and presence of concurrent disease infection (Khalafalla et al., 2010; Kihu et al., 2015). None of the samples was found positive for genome corresponding to the nucleoprotein gene. Though further investigations in camels are needed, one of the potential reasons may be the fact that PPR virus-induced viraemia is short-lived and virus does reside somewhere else in the body tissues. This is important because, despite exhibition of clinical picture corresponding to PPR infection in its primary host (the goat), genome corresponding to PPR virus was either not detected or detected in blood in a very low concentration among experimental animals (Bamouh et al., 2019). We used *N* gene specific PCR amplification simply because, among the molecular markers across the whole length of viral genome, it is considered the most replicative of the virus genes and the sequence and residue characteristics provide a distinctive resolution for PPRV epidemiology in disease endemic countries (Muniraju et al., 2014; Parida et al., 2015). Although the detection of PPR antibodies with absence of viral nucleic acids in camels in this study may not be an indicative of clinical disease, it remains to be investigated whether the existence of the virus in camels has any epidemiological significance for persistence of virus and subsequent transmission to other susceptible animal species.

We found a seroprevalence of 6.65% for brucellosis in camels. This is expected because the disease is endemic in the country with multiple evidences on varying rate of prevalence in human, small and large ruminants (Shafee et al., 2011; Arif et al., 2018; Saeed et al., 2019) and camels (Fatima et al., 2016; Shahzad et al., 2017). Despite zoonotic implications and potential of *Brucella* spp. to target a wide range of hosts (Musa et al., 2008; Khamesipour et al., 2014), brucellosis in camels has not received much attention worldwide. Camels are not believed to be a primary host of disease; however, when kept in a mixed farming system with small and large ruminants, they have a potential to play a significant role in cross-species transmission of brucellosis including humans (Rhodes et al., 2016; Viana et al., 2016), while sharing same vicinity for water and pasture (Teshome et al., 2003). A varying disease prevalence has also been evidenced in other camel rearing settings worldwide; however, rate of seroprevalence has been found to be relatively low (2–5%) in countries where camels are kept as nomadic pastoralists/agro-pastoralists, compared to those where they are kept intensively in a mixed animal population (8–15%) (Al-Khalaf and El-Khaladi, 1989; Radwan et al., 1995, 1992; Abbas and Agab, 2002), such as the studied herds in Pakistan.

MMultiple species of *Brucella* with a varying rate of prevalence have been evidenced from camel herds across many parts of the globe (Hamdy and Amin, 2002; Dawood, 2008; Khamesipour et al., 2014; Viana et al., 2016). A few of the examples include *B. melitensis* in Libya (Gameel et al., 1993), Egypt (Abdelgawad et al., 2017) and Saudi Arabia (Memish et al., 2002), and *B. abortus* in Sudan (Yagoub et al., 1990). Despite the fact that 1) we used a well-validated assay that can simultaneously detect and differentiate presence of particular *Brucella* spp. in a given sample, and 2) the presence of multiple *Brucella* spp., particularly *B. abortus* and *B. melitensis,* is being speculated in the susceptible animal population including camels in Pakistan without any laboratory-based evidence, we found a total of 19 herds (25.7%) exclusively infected with *B. abortus*. This corresponds to previous observations, where only *B. abortus* was reported in seropositive sheep and goat (Ali et al., 2015) and camel population (Fatima et al., 2016) in Pakistan. However, a caution in this regard must be considered, because detection of the bacterium may vary according to a particular

body fluid or tissue, and therefore there is a need for future investigations. For instance, a varying rate of detection of *B. abortus* and *B. melitensis* was reported for blood sample (4.1% vs. 2.4%) and lymph node (3.3% vs. 1.6%), respectively (Khamesipour et al., 2014). In another study, again using blood and lymph nodes, genome corresponding to *Brucella abortus* was detected in blood (2.2%) and *Brucella melitensis* in lymph nodes (3.0%) exclusively (Khamesipour et al., 2015). Hence, with these variable outcomes depending upon type of tissue to be used for antigen detection, another suggested thing to do in future is an antibody-based screening in camel population using a highly specific and sensitive assay corresponding exclusively to *B. melitensis*.

A number of categorical variables were significantly associated with an increased seropositivity of BT, PPR and brucellosis in camels in this study. These included pregnancy status (non-pregnant), mixed farming system, and breed (Desi) for BT, intensive or mixed feeding for PPR, and body condition and breed (Desi) for brucellosis. In a previous study from India, a high seroprevalence of BT in Desi breeds of camels has been evidenced (Chandel et al., 2003). Similarly, an increased odds of seropositivity were found for buffalo breed, large ruminants (cattle and buffalo) with a history of abortion (T. Sohail et al., 2019), and breed-type, parity, transhumant nature of animals and presence of vector for goat (Sohail et al., 2018). The odds of occurrence of PPR have been found varying in different geographical settings. For instance, in a study from Nigeria, age and body condition was found to be associated with the seropositivity in camels (Woma et al., 2015). Another study reported pregnancy, recent calving and old-age as risk factor for an increased seropositivity (Khalafalla et al., 2010). Although a number of potential risk factors are identified for an increased seropositivity of PPR, BT and brucellosis in animals other than camels, such as transboundary animal movements, introduction of new animal in a flock, environmental factors (increased temperature, humidity and rainfall), lack of awareness about disease, role of wildlife, illegal vaccination, lack of vector or other appropriate control measures etc., it is difficult to correlate observations reported in studies involving camels simply because the susceptibility of pathogen varies at large for small/large ruminants and camels.

This study had a few limitations. For instance, pooled sera were used for detection of specific pathogen through multiplex PCR, RT-PCR and rt-PCR. The pooling of samples was unavoidable due to resource limitations precluding the use of the commercial kit on samples from a large number of individuals. Although rt-PCR assay is considered more sensitive than conventional PCR, a use of standard PCR for brucellosis and PPR, and rt-PCR for BTV was associated to the availability of assay for each of these pathogens under limited resources in the laboratory. A random selection of animals was a consequence of the fact that selection of animals for resampling based on preliminary disease prevalence data was not practicable because most of the animal owners were nomadic and there exist an absolute lack of animal identification/tagging system. All camels included in this study were apparently healthy at the time of sampling and, according to the owners, none of them had previously shown clinical signs of brucellosis, BT and PPR, or at least they were not aware of it. Hence, these results indicate the disease carrying potential of camels posing a serious concern to veterinary and public health, and subsequent food insecurity. A continuous disease surveillance and appropriate interventions, particularly the vaccination of camel population, is suggested for an effective control of brucellosis, BT and PPR in the region.

CRediT authorship contribution statement

Muhammad Zubair Shabbir: Conceptualization, Data curation, Investigation, Formal analysis, Writing - review & editing. **Tayyebah Sohail:** Investigation, Formal analysis, Writing - review & editing. **Aziz Ul-Rahman:** Data curation, Investigation, Formal analysis, Writing - review & editing. **Tariq Abbas:** Investigation, Formal analysis. **Qasim Ali:** Data curation, Investigation. **Zia Ur Rehman:** Data curation. **Iahtasham Khan:** Resources. **Tahir Yaqub:** Conceptualization, Data curation, Resources. **Javed Muhammad:** Data curation. **Sohail Ahmad Khan:** Data curation. **Muhammad Saeed Imran:** Data curation. **Muhammad Abdul Basit:** Data curation. **Saleem Ullah:** Data curation. **Momena Habib:** Investigation, Formal analysis. **Syeda Anam Masood Gardezi:** Investigation. **Attia Bashir:** Formal analysis. **Mushtaq Ahmad:** Data curation, Resources. **Muhammad Abubakar:** Data curation, Resources. **Muhammad Abbas:** Data curation, Resources. **Atta Subhani:** Data curation. **Ghulam Yasein:** Data curation. **Fariha Altaaf:** Investigation. **Muhammad Hasaan Aziz:** Data curation, Investigation. **Riaz Hussain:** Resources. **Ali Zohaib:** Resources. **Umer Chaudhry:** Conceptualization, Investigation. **Jonas Johansson Wensman:** Conceptualization, Resources, Writing - review & editing.

Declaration of Competing Interest

None

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2020.105435.

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