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Sero-Epidemiological Investigation of Abortifacient Bacteria in Goats and Sheep in Three Districts of Sindh Province of Pakistan

Asadullah Memon[1], Asghar Ali Kamboh[1]*, Saeed Ahmed Soomro[2], Muhammad Ammar Khan[3], Akeel Ahmed Memon[4] and Hubdar Ali Kolachi[1]

[1]Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan
[2]Department of Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan
[3]Department of Food Science and Technology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[4]Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan

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Key words
Q fever, *Coxiella burnetiid*, Chlamydiosis, Brucellosis, Sheep, Goat

ABSTRACT

Abortifacient bacteria incur high production and economic losses in small ruminants worldwide. The present study assessed the prevalence of *Coxiella burnetii, Chlamydia abortus,* and *Brucella melitensis* in 178 sheep and goat samples obtained from Hyderabad, Tando Allahyar, and Mirpurkhas districts of Sindh Province of Pakistan. The results revealed that the seroprevalence of Q fever was significantly (p<0.05) higher than chlamydiosis and brucellosis (43.82% vs. 37.7% and 17.98%). The districts-wise incidences of seroprevalence were also significant in this study, as *C. burnetii* antibodies were significantly (p<0.05) higher in Hyderabad than Tando Allahyar and Mirpurkhas (70.37% vs. 37.25% and 9.09%); *C. abortus* higher (p<0.05) in Mirpurkhas than Hyderabad and Tando Allahyar (100%, 40.74%, 21.56%, respectively); but *B. melitensis* higher (p>0.05) in Hyderabad than Tando Allahyar and Mirpurkhas (29.63%, 15.69%, 0%, respectively). The animal-wise seroprevalence results also exhibited significant differences (p<0.05), as the incidence of higher *C. abortus antibodies* (42.46% and 12.50%); but lower *B. melitensis* (10.95% and 50%) in goats than sheep sera, respectively. However, there was no clear relation between the breed of the ruminants and their seropositivity for *C. burnetii*, *C. abortus*, and *B. melitensis*. In addition, the seroprevalence of *C. burnetii* was higher (p<0.05) in nulliparous goats and sheep than multiparous and primiparous (37.50% and 50%, 35.89% and 40%, 30.76 and 25%, respectively). Moreover, *C. abortus* antibodies were higher (p<0.05) in multiparous goats and sheep than primiparous and nulliparous (48.71% and 20%, 38.46% and 0%, 25% and 0%, respectively). Furthermore, the incidence of *B. melitensis* antibodies was higher (p<0.05) in nulliparous than primiparous and multiparous goats (37.50%, 11.53%, and 5.12%, respectively), but higher (p<0.05) in primiparous than nulliparous and multiparous sheep (75%, 50%, 40%, respectively). Finally, the sera found positive on ELISA for *C. abortus, C. burnetii*, and *B. melitensis* antibodies. In conclusion, the widespread prevalence of the three abortifacient bacteria was responsible for the production and economic losses of goats and sheep in the three selected districts of Sindh Province of Pakistan.

INTRODUCTION

In does and ewes, annual abortion percentage estimated as high as 14% (Alemayehu *et al*., 2021), however, in Sindh province of Pakistan it is documented 12.5% (Memon, 2018). Productivity reduction in small ruminants, such as sheep and goats, due to reproductive disorders incurs adverse effects on the economy of rural people (Ishfaq *et al*., 2017). Fetus loss due to the infectious and non-infectious abortive agents is the most important reason for those losses (Mammeri *et al*., 2013). The most common causes of abortifacient infections include *Brucella melitensis*, *Coxiella burnetii*, and *Chlamydia abortus* pathogens (Al-Qudah *et al*., 2004; Benkirane *et al*., 2015). These are bigger threats than non-infectious agents, as they carry significant zoonotic risk to humans who take care of infected goats and sheep (Benkirane *et al*., 2015; Hazlett *et al*., 2013). Abortifacient infections

* Corresponding author: drasgharkamboh@yahoo.com
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collectively caused million dollars losses annually and may cut 65% of overall profit of the farmer (Gebretensay *et al*., 2019). Other hazardous pathogens which induce abortion in sheep and goats include *Listeria*, *Toxoplasma,* and *Leptospira* (Heidari *et al*., 2018). These also carry significant zoonotic risk, as they readily transmit among animals and humans during grazing (Tesfaye *et al*., 2020).

The infection of *C. abortus* is known as chlamydiosis, which is a communicable disease and causes negative effects on sheep breeding worldwide (Tesfaye *et al*., 2020). It has been estimated that in pregnant does chlamydia may cause abortion and birth of stillborn or weak kids up to 60% in naïve herds (Tibary, 2021). Chlamydiosis exhibits no clinical signs until abortion. The animal born is also very weak in case of successful delivery (Aitken and Longbottom, 2007). Chlamydia organisms belong to family *Chlamydiaceae* that has two genera, namely Chlamydophila and Chlamydia. Both these genera have nine species that are obligate intracellular parasites (Aitken and Longbottom, 2007).

Coxiella burnetii is the causative agent of Q fever that may infect up to 50% of sheep herds and results late term abortions, stillbirth and weak lambs (Tibary, 2021). Query fever also known as coxiellosis has been attributed as a cause of loss of billions of dollars worldwide (with exception of New Zealand) (Hechemy, 2012). It is a ubiquitous zoonotic disease that reported in several animals, including sheep and goats (Kelkay *et al*., 2017). *C. burnetii* is a Gram-negative, obligate intracellular pathogen that cause abortion, stillbirth, and early mortality of young ones without showing clear signs of disease in the mother animals (Abushahba *et al*., 2017). *C. burnetii* was first identified as a rickettsia-like organism, which was isolated from spleen and liver samples of mice captured from the abattoir (Selim *et al*., 2018).

Brucellosis is caused by *B. melitensis*, which is also recognized as a common abortifacient bacterium of farm animals. It causes abortion in small ruminants in the third trimester of their pregnancy in more than 27% animals (Samadi *et al*., 2010). The infected animal transmits this bacterium in milk, vaginal discharge, urine, and feces during infection. The mothers with this infection may become infertile in subsequent life (Selim *et al*., 2015).

In order to save the small ruminants from abortion-related losses, seroepidemiological investigations were largely carried out all over the world (Anastacio *et al*., 2013; Tesfaye *et al*., 2020; Traore *et al*., 2021). In the Punjab province of Pakistan, some studies (Zahid *et al*. 2016; Rashid *et al*. 2019; Ullah *et al*. 2019) were also carried out to know the prevalence of abortifacient pathogens in apparently healthy animals. To the best of our knowledge, no work is reported from Sindh province of Pakistan, thus this epidemiological study was conducted in sheep and goats in three districts (Hyderabad, Tando Allahyar, and Mirpurkhas) to know a clear picture of abortifacient bacteria in apparently healthy small ruminants in Sindh. This study would help the researchers and government bodies formulate strategic future plans to save the losses in small ruminants.

MATERIALS AND METHODS

Ethics statement

All experimental procedures were carried in line with the International Animal Ethics Guidelines. The study was conducted after the approval of the Directorate of Advanced Studies, Sindh Agriculture University, Tandojam, Sindh Province, Pakistan (No. DAS/2674 of 2019).

Animals and sampling

This study utilized a convenience sampling approach for the collection of sheep and goat samples. The total number of blood samples collected from three districts of Sindh province were 178 (Hyderabad, n=54; Tando Allahyar, n=102; and Mirpurkhas, n=22). The information regarding area, breed, parity, etc. of goats and sheep was also collected during the survey, which was linked with the help of statistical tools with the prevalence of abortifacient bacteria. Samples (blood) were collected from apparently healthy female sheep and goats without considering any abortion history. Samples were collected from both pure (descriptive) and non-pure (non-descriptive) sheep and goat breeds using jugular vein. Before collection of samples, the sampling area was shaved, cleaned with antiseptic solution and disposable syringes with a 16 G needle were used for blood (5 mL) collection. Samples were labeled and transported to the laboratory under cooling condition.

Processing of samples

Immediately after collection, blood samples were poured into 10 mL dry tubes. The tubes were placed at room temperature (25±2°C) for 24 h for serum separation. After this period, these were subjected to centrifugation at 1500 g for 10 min. Subsequently, the sera were stored at a temperature of -20°C until required for analysis. Commercially available ELISA (Enzyme-linked Immunosorbent Assay) kits were purchased and utilized to investigate the seroprevalence of *C. abortus* (Kit No. CLA1135T, IDEXX-Germany), *C. burnetii* (Kit No. QFT1135T, IDEXX-Germany), and *B. melitensis* (Kit No. P04130-10, IDEXX-Germany). According to the manufacturer's instructions sera samples were investigated in microtitre plates coated with inactivated antigen that were read using microplate reader (Bio-Rad, UK) at wave length of 450 nm. The data were analyzed by putting the optical density (OD) values of test sample (OD_S), negative (OD_{NC}) and positive controls (OD_{PC}) in the following formula.

$$S/P= [(OD_{S} - OD_{NC}) / (OD_{PC} - OD_{NC}) \times 100]$$

The results were interpreted as follow: values less than 30% were considered negative, ≥40% were considered positive, while those between 30-40% were considered suspected. Whereas, we simplified and S/P values ≥40% were considered positive (and considered for risk factor analysis) while those have less than 40% were considered negative.

Statistical analysis

Microsoft Excel was used to calculate the mean values of the data, while the Chi-square test was used to calculate the risk factors using JMP statistical package software (version 5.0.1.a, SAS Institute Inc., Cary, NC). Odds ratio (OR) was determined for all the risk factors related to the seropositivity of Q fever, chlamydiosis, and brucellosis. Confidence level at 95% was determined and $p<0.05$ was used as significance level in all statistical analyses.

RESULTS

Overall prevalence

Significant differences ($p<0.05$) were observed among the seroprevalences of Q fever, chlamydiosis, and brucellosis in this study (Fig. 1). The highest seroprevalence of 43.82% was recorded in the case of Q fever, which followed by 37.7% for chlamydiosis and 17.98% for brucellosis.

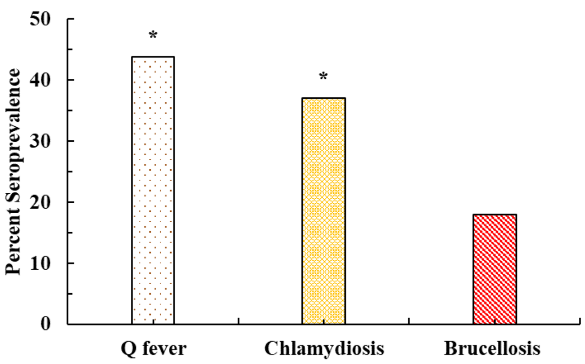

Fig. 1. Overall prevalence of abortion causing diseases in the study area. * Statistically higher at p < 0.05.

Seroprevalence of Q fever (C. burnetii)

The district-wise data obtained from the three selected districts of Sindh Province of Pakistan showed that the differences in the prevalence of *C. burnetii* antibodies were significant ($p<0.05$) among the selected districts (Table I). The highest ($p<0.05$) prevalence was observed in the case of district Hyderabad (70.37%), followed by Tando Allahyar (37.25%, OR: 0.5), and Mirpurkhas (9.09%, OR: 0.1). The animal-wise data of the sera also exhibited that the differences were nonsignificant ($p>0.05$), with numerically higher seroprevalence in goats (45.20%) as compared to sheep (37.50%). The parity-wise data in this study also revealed significant ($p<0.05$) differences among nulliparous, multiparous, and primiparous goats and sheep. The highest ($p<0.05$) prevalence was observed in the case of nulliparous (37.50%), followed by multiparous (35.89%, OR: 0.9), and primiparous (30.76%, OR: 0.8) goats. Similarly, the highest ($p<0.05$) prevalence was observed in the case of nulliparous (50%), followed by multiparous (40%, OR: 0.8), and primiparous (25%, OR: 0.5) sheep. The population-wise data also showed nonsignificant ($p>0.05$) differences among descript and nondescript goats and sheep, with higher seroprevalence for descript goats and sheep (35.89% and 40%, respectively) as compared to nondescript goats and sheep (32.35% and 33.33%, respectively).

Table I. Seroprevalence of Q fever in sheep and goats.

Variable/ category	No. of +ve samples (%)	Odds ratio	95% CI	p value
Area (District)				
Hyderabad	38 (70.37)	1.0	63.29-85.10	0.0298
Tando Allahyar	38 (37.25)	0.5	33.37-41.09	
Mirpurkhas	2 (9.09)	0.1	4.76-14.02	
Individual prevalence				
Goat	66 (45.20)	1.0	42.29-49.70	0.0833
Sheep	12 (37.50)	0.8	32.77-41.25	
Parity (goat)				
Nulliparous	6 (37.50)	1.0	25.81-51.93	0.0374
Primiparous	16 (30.76)	0.8	25.97-34.46	
Multiparous	28 (35.89)	0.9	32.46-39.61	
Parity (sheep)				
Nulliparous	2 (50)	1.0	23.47-75.56	0.0398
Primiparous	2 (25)	0.5	9.07-40.59	
Multiparous	8 (40)	0.8	35.65-46.29	
Breed (Goat)				
Descriptive	28 (35.89)	1.0	24.67-43.05	0.0833
Non descriptive	22 (32.35)	0.9	28.78-37.77	
Breed (Sheep)				
Descriptive	8 (40)	1.0	35.36-45.88	0.0849
Non descriptive	4 (33.33)	0.8	29.94-37.27	

Seroprevalence of Chlamydiosis (C. abortus)

In current study, the district-wise data obtained from the three selected districts of Sindh Province of Pakistan showed that the differences in the prevalence of *C. abortus* antibodies were significant ($p<0.05$) among the selected districts (Table II). The highest ($p<0.05$) prevalence was observed in the case of district Mirpurkhas (100%) with 2.5 times higher likelihood of disease, followed by Hyderabad (40.74%), and Tando Allahyar (21.56%). The animal-wise data of the sera also exhibited that the differences were significant ($p<0.05$), with higher seroprevalence for goats (42.46%) as compared to sheep (12.50%, OR: 0.3). The parity-wise data in this study revealed significant ($p<0.05$) differences among nulliparous, multiparous, and primiparous goats only. The highest ($p<0.05$) prevalence was observed in the case of multiparous (48.71%), followed by primiparous (38.46%, OR: 1.3), and nulliparous (25%, OR: 1.9) goats. However, the numerically higher ($p>0.05$) prevalence was observed in case of multiparous (20%), followed by primiparous and nulliparous (0%) sheep. The population-wise data also showed nonsignificant ($p>0.05$) differences among descript and nondescript goats and sheep, with numerically higher seroprevalence for descript goats and sheep (48.71% and 20%, respectively) as compared to nondescript goats and sheep (35.29% and 0%, respectively).

Seroprevalence of brucellosis (B. melitensis)

The district-wise data obtained from the three selected districts of Sindh Province of Pakistan showed that the differences in the prevalence of *B. melitensis* antibodies were not significant ($p>0.05$) among the selected districts (Table III). The highest ($p<0.05$) prevalence was observed in the case of district Hyderabad (29.63%), followed by Tando Allahyar (15.69%), and Mirpurkhas (0%). In contrast, the animal-wise data of the sera exhibited that the differences were significant ($p<0.05$), with 5 times higher seropositivity in sheep (50%) as compared to goats (10.95%). The parity-wise data in this study also revealed significant ($p<0.05$) differences among nulliparous, multiparous, and primiparous goats and sheep. The highest ($p<0.05$) prevalence was observed in the case of nulliparous (37.50%), followed by multiparous (11.53%, OR: 0.3), and nulliparous (5.12%, OR: 0.1) goats. Similarly, the highest ($p<0.05$) prevalence was observed in the case of primiparous (75%) with 1.5 times higher risk of disease, followed by nulliparous (50%), and multiparous (40%) sheep. The population-wise data showed nonsignificant ($p>0.05$) differences among descript and nondescript goats and sheep, with numerically higher seroprevalence for nondescript goats (17.64%) as compared to descript goats (5.12%), as well as higher seroprevalence for nondescript sheep (66.66%) as compared to descript sheep (40%).

Table II. Seroprevalence of Chlamydiosis in sheep and goats.

Variable/ category	No. of +ve samples (%)	Odds ratio	95% CI	p value
Area (District)				
Hyderabad	22 (40.74)	1.0	34.45-46.68	0.0031
Tando Allahyar	22 (21.56)	0.5	19.98-24.42	
Mirpurkhas	22 (100)	2.5	87.39-108.68	
Individual prevalence				
Goat	62 (42.46)	1.0	39.08-46.83	0.0192
Sheep	4 (12.50)	0.3	7.47-18.58	
Parity (goat)				
Nulliparous	4 (25)	1.0	17.85-35.92	0.0267
Primiparous	20 (38.46)	1.3	34.34-41.38	
Multiparous	38 (48.71)	1.9	44.48-53.64	
Parity (sheep)				
Nulliparous	0 (0)	1.0	-	1.0000
Primiparous	0 (0)	1.0	-	
Multiparous	4 (20)	20	-19.23-34.59	
Breed (Goat)				
Descriptive	38 (48.71)	1.0	39.83-57.29	0.4601
Non descriptive	24 (35.29)	0.7	27.72-43.92	
Breed (Sheep)				
Descriptive	4 (20)	1.0	8.29-27.44	0.2888
Non descriptive	0 (0)	0.0	-	

DISCUSSION

The population of sheep and goat is 29,789 and 70,724 thousands in Pakistan, while in Sindh it is 3959 and 12,571 thousands, respectively. The share of livestock is accounted 11.11% in national GDP and 58.92% in agriculture (GOP, 2021). These animals are the basic livelihood of millions of people. Many infections can lead to abortion in goats and sheep, which are the causes of significant productivity and economic loss to livestock farmers. The seroprevalence of antibodies against abortifacient bacteria such as Q fever, chlamydiosis, and brucellosis in Sindh Province of Pakistan has not been previously published. The present study is the first scientific report on seroprevalence of abortion-causing bacteria in apparently healthy small ruminants in selected three districts of Sindh Province of Pakistan, including Hyderabad, Mirpurkhas, and Tando Allahyar. These three districts have a dry hot climate around the year and have been situated on the East of River Indus. These districts have abundant herds of livestock including small ruminants.

Table III. Seroprevalence of Brucellosis in sheep and goats.

Variable/ category	No. of +ve samples (%)	Odds ratio	95% CI	p value
Area (District)				
Hyderabad	16 (29.63)	1.0	12.25-43.35	0.2181
Tando Allahyar	16 (15.69)	0.5	10.63-21.59	
Mirpurkhas	0 (0)	0.0	-	
Individual prevalence				
Goat	16 (10.95)	1.0	7.50-13.73	0.0050
Sheep	16 (50)	5.0	43.58-60.92	
Parity (goat)				
Nulliparous	6 (37.50)	1.0	30.54-44.64	0.0386
Primiparous	6 (11.53)	0.3	9.91-13.62	
Multiparous	4 (5.12)	0.1	4.11-6.54	
Parity (sheep)				
Nulliparous	2 (50)	1.0	42.21-59.78	0.0291
Primiparous	6 (75)	1.5	64.46-87.47	
Multiparous	8 (40)	0.8	34.44-45.46	
Breed (Goat)				
Descriptive	4 (5.12)	1.0	-1.26-12.92	0.1269
Non descriptive	12 (17.64)	3.4	11.76-24.43	
Breed (Sheep)				
Descriptive	8 (40)	1.0	29.43-49.88	0.5582
Non descriptive	8 (66.66)	1.6	46.67-82.81	

The overall seroprevalence results of *C. abortus, C. burnetii,* and *B. melitensis* in this study showed that about one-third (33.16%) of the total population of small ruminants has antibodies against abortifacient bacteria, which indicates a high infection level in sheep and goats of the study area. A similar percentage of seroprevalence of abortifacient bacteria was previously reported by Schnydrig *et al.* (2017), who observed that about 31.2% of small ruminants in Switzerland were infected by *C. abortus, C. burnetii, B. melitensis,* and *Leptospira* spp. Even higher incidence of abortifacient bacteria was reported by Heidari *et al.* (2018), who observed that about 47% of small ruminants in Iran were infected by *C. abortus, C. burnetii,* and *Mycoplasma agalactiae*.

The prevalence status of *C. burnetii* was previously investigated and confirmed in the range of 6.1 to 30.8% in the Punjab province of Pakistan by Ahmad (1987), Shabbir *et al.* (2016), Zahid *et al.* (2016), Rashid *et al.* (2019) and Ullah *et al.* (2019) in different animal species. The results of seroprevalence of *C. burnetii* antibodies in Sindh Province, which accounted for about 45.20% in goats and 37.50% in sheep in this study, were higher than those reported in Punjab. The incidence of *C. burnetii* was 28.46% in Ethiopia in small ruminants (Tesfaye *et al.*, 2020), which was lower than the present study. These results confirmed the findings of Zahid *et al.* (2016) who conducted their study in Pakistan, and Gache *et al.* (2017) who conducted their study in France. In some other studies, opposite results were observed, as Kshash (2012) and Anastacio *et al.* (2013) reported higher seroprevalence of *C. burnetii* in sheep as compared to goats. These contrasting trends were previously attributed to malpractices of animal management (Zahid *et al.* 2016), and harsh climate including prevalent dusty winds (van der Hoek *et al.*, 2011), because no evidence of intrinsic vulnerability was found for any particular small ruminant in any previous study. These results were further strengthened by differences found in seropositive cases in different districts in the current study.

In current study, more than one-third (37.07%) population of goats and sheep in the three selected districts was found seropositive for *C. abortus*, with high rate in goats (42.46%) than those of sheep (12.5%). These results were quite different from those reported by Al-Qudah *et al.* (2004), who found a lower incidence of *C. abortus* in goats (11.4%) but higher in sheep (21.8%) in Jordon. Similarly, the results of the present study were also in contrast with those reported by Schnydrig *et al.* (2017), who found a lower incidence of *C. abortus* in caprine sera (26.7%) than in the present study, but higher in ovine sera (38.1%) in Switzerland. The difference in the results of this study could be attributed to the different gene pool, inherent resistance of the animals, environment, district (Liu *et al.*, 2005), analytical method (Qin *et al.*, 2015), and the number of samples of a particular breed. The incidence of *C. abortus* in this study was also different in each district selected in this study. This trend is in coincidence with different results of chlamydiosis in different areas, as reported by Al-Qudah *et al.* (2004) for 11.2% in Ajloun versus 31.2% in Mafraq areas. The trend was also in agreement with the results of chlamydiosis infection in ovine sera in Sertao (34.6%) and East region (13.2%) in Brazil (Pinheiro *et al.*, 2010). A human study also exists that showing the role of parity and gonorrhoeal infection, while area was not recognized as risk factors for chlamydiosis (Liu *et al.*, 2013). Type of animal is also an important factor besides area, as the incidence of *C. abortus* was quite high in sheep (13 out of 13 flocks) and goats (8 out of 10 flocks) in the Middle Atlas and Northern

Morocco (Benkirane *et al*., 2015).

The incidence of Brucellosis in the present study was 17.98%. The present study was conducted in three districts of Sindh province of Pakistan, while these results were higher than those reported in Baluchistan (2.3%), Punjab (7%), and KPK (10%), which are other provinces of Pakistan (Shafee *et al*., 2016). The differences of the present research with these studies could be attributed to differences in experimental design, sampling method, analytical technique, area, and climate, etc., (Durrani *et al*., 2015; Soomro *et al*., 2014). The seroprevalence results of current study were also inconsistent with results of two different studies reported from India i.e., 5.9% in small ruminants of organized farming of Gujarat state (Kanani *et al*., 2018), and 7.45% in a nation-wide study (Shome *et al*., 2020). These results were, however, much higher than Ethiopia (0%) in small ruminants (Tesfaye *et al*., 2020). The above results also revealed that the incidence of *Brucella* infection was affected by the geographical location. Moreover, sera of sheep exhibited significantly higher *Brucella* antibodies than in goats, which was in the coincidence of the findings of Saeed *et al*. (2019). Parity also significantly affected the brucellosis prevalence in small ruminants in this study, which was in agreement with the findings of Gul *et al*. (2015). The effect of parity could be attributed to the fact that repeated exposure to parturition and other physiological stresses during gestation may significantly affect the inherent resistivity of animals against infectious organisms. However, an earlier study found no effect of parity, species, and pregnancy status on the seropositivity of brucellosis (Kelkay *et al*., 2017).

CONCLUSIONS

This study was conducted to investigate the extent of Q fever, chlamydiosis, and brucellosis in small ruminants of Hyderabad, Tando Allahyar, and Mirpurkhas districts of Sindh Province of Pakistan. The seroprevalences of Q fever and chlamydiosis were higher than that of brucellosis. Moreover, the seroprevalences of Q fever and chlamydiosis were higher in goats than sheep but higher in sheep than goats in the case of Brucellosis. Finally, animal breed had no association with the seropositivity of all the abortion-causing bacteria investigated in this study. The present investigation will serve as an index of the prevalence of abortifacient bacteria in Sindh province of Pakistan, hence it will help regulatory bodies to establish preventive and protective measures to reduce economic and productivity losses related to the livestock of Pakistan.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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