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Serological Evidence of Chlamydiosis, Q Fever and Brucellosis in District Dadu, Pakistan

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ABSTRACT

Brucella melitensis, Chlamydophila (=*Chlamydia*) *abortus* and *Coxiella burnetii* are common abortifacient bacterial pathogens in sheep and goats. The establishment of active animal surveillance is essential to detect incidences as outbreaks of infections in small ruminants. The current study was planned to determine presence of these bacterial organisms causing abortions in goats and sheep in district Dadu, Sindh, Pakistan. A total of 224 samples (blood= 184, vaginal swab= 05, foetal membrane=02 and milk=33) were obtained from the selected animals. The health status, age, sex, parity and history of abortion for each goat and sheep was documented. The serological test I-ELISA and biochemical reactions were performed for detection of *Brucella melitensis*, *Chlamydia abortus* and *Coxiella burnetii*. The antibiogram of the isolated *B. melitensis* was performed using Kirby Baur Disk Diffusion method. On serological analysis of 184 blood samples, 118 (64.13%) samples were found positive for the abortifacient bacteria. Out of these 184 samples, 67 (36%), 47 (25%) and 4 (2.17%) samples were positive for *C. abortus*, *C. burnetii* and *B. melitensis*, respectively. Sixty-seven samples, caprine 34 (37.3%) and ovine 33 (35.3%), were found positive for *C. abortus*. Forty-seven samples, does 31 (33.33%) and ewes 16 (16.9%) were detected positive for *C. burnetii*. Only 3 (3.7%) samples from goats and 01 (1.5%) sheep samples were found positive to *B. melitensis*. There is no significant difference in the comparative prevalence of the three major abortive infection (*C. burnetii*, *C. abortus* and *B. melitensis*) among nulliparous, primiparous and multiparous goats and sheep. Out of 40 samples cultured (vaginal swab= 05, foetal membrane=02 and milk=33), 5 samples were found positive for *B. melitensis*. The antibiogram indicated *B. melitensis* isolates were highly susceptible to oxytetracycline, doxycycline and ciprofloxacin. While the organism found resistant to erythromycin, lincomycin, rifampicin, cephalothin, bacitracin, streptomycin and gentamycin. In conclusion, *Brucella melitensis, Chlamydophila abortus* and *Coxiella burnetii* infections were prevalent in different areas of district Dadu, Sindh, Pakistan. *B. melitensis* was highly sensitive to oxytetracycline, doxycycline and ciprofloxacin.

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Authors' Contribution
HAK carried out the experiment. SHA acted as a major supervisor of the project. AAK and SAS conceived the study design and analyse the data. NHK and AAS helped in writing of manuscript.

Key words
Q fever, Chlamydiosis, Brucellosis, Small ruminants, Sindh

INTRODUCTION

The first species of animals to be domesticated by human beings were sheep and goats. Sheep and goats constitute approximately half of the domesticated ruminants population in the world (Jesse *et al*., 2020). The global population of these animals have been increased from 1.35 billion to 1.94 billion (Solangi *et al*., 2023). About 90% of caprine farmed in the developing countries, are one of the valuable sources of protein for humans (Tyasi *et al*., 2020). In certain countries, small ruminants can be considered as "liquid asset" in h of need (Teklebrhan *et al*., 2014). According to economic survey of Pakistan, 2021-2022 contribution of livestock in agriculture sector is 61.9% and 14. 0% in overall GDP of Pakistan. Pakistan owns vast number of small ruminant population with an estimated number of 82.5 and 31.9 million heads of goats and sheep, respectively (Economic Survey of Pakistan, 2022).

Abortion in goats and sheep has worldwide importance because of crucial economic losses including zoonotic risk (Benkirane *et al*., 2015; Van Engelen *et al*., 2014). In the

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year 2016, 1275 miscarriage cases in sheep and 1143 in goats were reported in Jordan. Reproductive disorders have negative effect on farm animal outcomes, health and peoples economy (Chu *et al*., 2016; Van Engelen *et al*., 2014).

Various infectious and noninfectious agents are potential cause of abortion. Abortion due to pathogenic organisms usually takes place during the last three months of gestation (Holler, 2012). While noninfectious causes are phytotoxins, dietary deficiencies of micro minerals (copper, selenium, magnesium), vitamins, hormones (cortisol, estrogen), phenothiazine and CCL_4 (Tramuta *et al*., 2011). There are number of infectious organisms such as *Toxoplasma gondii*, *Brucella melitensis*, *Listeria* spp., *Chlamydia abortus, Coxiella burnetii,* blue tongue virus, foot-and-mouth disease virus, small pox and *Campylobacter fetus,* which cause abortion in domesticated animals (Van den Brom *et al*., 2015; Ababneh *et al*., 2014; Abousenna *et al*., 2020; Jabary *et al*., 2020).

Chlamydophila (=*Chlamydia*) *abortus, Brucella melitensis,* and *Coxiella burnetii* are bacterial pathogens causing abortion (Ganter, 2015). In addition to these some other opportunistic pathogens also responsible for abortion (Vidal *et al*., 2016). The diseases caused by these organisms may lead to congenital defects, loss of foetus and abortion in ruminants (Van Engelen *et al*., 2014). Livestock owners in different districts of Sindh have reported higher abortion rates causing economic losses in small ruminants but the cause remained a mystery (Memon *et al*., 2022). Therefore, the establishment of active animal surveillance is essential to detect incidences as outbreaks of infections. Because of the impact of such infections on production and economic losses in livestock, the study was conducted to detect different bacterial organisms causing abortions in small ruminants and their prevalence in district Dadu, Sindh, Pakistan.

MATERIALS AND METHODS

The study design was based on abortion cases in the district Dadu, Sindh Pakistan, on the basis of the presence of susceptible clinical cases of abortion in small ruminants and previous evidence of abortions in goats and sheep. The study was conducted in ten villages of Dadu district that represented all four talukas of Dadu district viz., Dadu, Khaipur Nathan Shah, Mehar and Johi (Fig. 1). Dadu has rich alluvial loamy soil of the Indus valley and has high number of small ruminants, but overall Dadu district is economically backward area of Sindh province. Selection of villages was based on information collected through survey form. A total of 224 samples were taken from the selected animals using random-sampling approach. The health status, age, sex, parity and history of abortion for each goats and sheep was documented. In addition, the history of farming personals in relation to abortion, dystocia, infertility, still birth or any other reproductive disorder data was obtained.

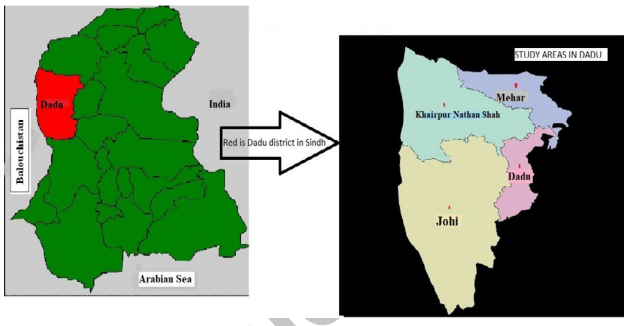

Fig. 1. Map of study area of Sindh province of Pakistan.

In this investigation, a total of 224 (n=184 for serology and n= 40 for culture) samples were investigated for the presence of abortifacient bacteria. A total number of 112 goats were selected for sampling from Monder village, Dadu city, Khairpur Nathan Shah and Kakar areas. Whereas the 112 sheep samples were collected from in and around areas of Kakar and Mujaver villages of Khairpur Nathan Shah district Dadu. The samples were comprised of blood, milk, vaginal swab and placenta.

Collection of samples

Blood samples were collected from jugular vein of goats and sheep in 5ml sterile plain vacutainers aseptically. After collection samples were kept on ice and transported to the Department of Veterinary Microbiology, Sindh Agriculture University, Tandojam. The specimens were centrifuged at 10,000 rpm for 5 min to separate the sera. The sera were carefully transferred to sterile 3 ml Eppendorf tubes. The sera samples were stored at -20°C for further investigations.

Vaginal swab, foetal membrane and milk specimens were also collected from the ruminants with recent history of abortion or those aborted at the time of sampling in selected areas. Vaginal swabs samples were collected by culture swabs in sterile tubes containing transported medium sucrose phosphate glutamate (SPG) buffer and brought to the laboratory of Department of Veterianry Microbiology for further processing. Milk samples were aseptically collected from lactating goats and sheep by hand stripping using sterile screw capped 25ml tubes. The samples were transported to the Central Veterinary Diagnostic Laboratory Tandojam in ice bags and kept in refrigerator till processed for bacterial isolation using

Brucella selective agar (Hi Media, India).

Serological assay

The serological test I-ELISA to detect *Brucella melitensis*, *Chlamydia abortus* and *Coxiella burnetii* was carried out at Sindh Poultry Vaccine Centre, Karachi, Sindh, using commercial kits of Enzyme linked immunosorbent assay (ELISA). All test procedures were carried out according to manufacturers' instruction (IDEXX-Germany) for the use of *B. melitensis* (catalogue number P04130-10), *C. burnetii* (catalogue number QFT1135T) and *C. abortus* (catalogue number CLA1135T) kits.

Isolation of Brucella *from milk and swab samples*

Isolation and identification of *Brucella* was carried out as per OIE Manual Standard Procedure (2013). Forty clinically suspected samples of milk (n=33), placenta (n=2) and vaginal swab (n= 5) samples were inoculated on Brucella Selective Agar (Hi Media, India). The inoculated plates with specimen were incubated at 37°C for up to 4 days and colonies were checked after every 24 h. *Brucella* assumed colonies were picked up by sterile wire loop, mixed with a drop of distilled water on a clear glass slide to make smear. Then the smear was heat fixed and dried in an incubator. The slide was stained by Ziehl–Nielsen's (differential stain) method. *Brucella* species identification was based on Gram negative, pin point smooth appearance and coccobacilli shape arranged mostly in single but some in pairs and in bunches according to Alton *et al*. (1988).

Antimicrobial susceptibility testing

The antibiogram of isolates was carried out using prescribed method of disk diffusion by Bauer *et al*. (1966). The bacterial standardized pure culture inoculum was swabbed onto surface of specific Mueller Hinton agar and *Brucella* selective agar. Filter paper disks impregnated with antimicrobial agents were placed on the media plates. After overnight incubation at 37 °C, the zones of inhibition were measured.

Statistical analysis

All collected data was entered Excel (Microsoft office, 365) spread sheets to calculate the means. Then it was transferred to statistix software (Version 8.1, 1985-2005 Analytical Software.) to calculate the risk factors using Chi-square test.

RESULTS

Sero-prevalence of the abortifacient bacteria in goats and sheep

On analysis of the 184 serum samples of ovine and caprine thorough I-ELISA, the total seroprevalence of common abortifacient bacteria obtained was 64.13%. 118 samples were found positive out of 184 samples (Table I). Individual pathogen wise, out of 184 serum samples 67 (36%), 47 (25%) and 4 (2.17%) were serologically positive for *Chlamydia abortus*, *Coxiella burnetii* and *Brucella melitensis*, respectively. The prevalence percentage of *C. abortus* found in does 34 (37.3%) was higher than ewes 33 (35.3%). Similarly, *C. burnetii* prevalence was higher in does 31 (33.33%) than ewes 16 (16.9%). Four samples (2.17%) were found positive for *B. melitensis*. Out of these four samples, 3 (3.7%) were detected positive from goats, while one sample (1.5%) from sheep was found positive (Table I).

Association of risk factors with abortifacient bacteria in small ruminants

The distribution of various abortifacient bacteria according to the parity (nulliparous vs primiparous vs multiparous) and abortion frequency (once vs ≥ twice) was analyzed and presented in Table II. There is no significant difference in the comparative prevalence of the three major abortive infection (*C. burnetii*, *C. abortus* and *B. melitensis*) among nulliparous, primiparous and multiparous goats and sheep. The Chi square calculations also showed no significant difference in these three abortive bacteria between once and ≥ twice aborted goats and sheep.

Brucella melitensis *in milk and swab samples*

In present study, *Brucella melitensis* was isolated from the milk and vaginal swabs. Out of total 40 samples 20% (01) from vaginal swab and 12.12% (04) from milk samples were found positive, while no isolates were obtained from placental samples of aborted animals (Table III).

Table I. Seroprevalence of abortifacient bacteria in goats and sheep in district Dadu, Sindh.

Animal species	Samples tested	Positive for *C. burnetii*	Positive for *C. abortus*	Positive for *B. melitensis*	Total positive	x^2 (d.f)	*p*-value
Goats	92	31 (33%)	34 (37.3%)	3 (3.7%)	68 (73.91%)	3.13(2)	0.2092
Sheep	92	16 (17%)	33 (35.3%)	1 (1.5%)	50 (54.34%)		
Total	184	47 (25%)	67 (36%)	4 (2.1%)			

Table II. The occurrence of abortifacient bacteria in relation to parity and abortion frequency in small ruminants.

Variable	No. of animals	*C. burnetii*	*C. abortus*	*B. melitensis*	x^2(d.f)	p-value
Parity						
Nulliparous	26	4 (15.38)	7 (26.92)	0 (0)	9.11 (4)	0.06
Primiparous	58	9 (15.51)	10 (17.24)	3 (5.17)		
Multiparous	100	34 (34)	50 (50)	1 (1.00)		
Total	184	47	67	04		
Abortion frequency						
Once	168	40 (23.8)	58(34.52)	03(1.78)	0. 42(2)	0.8087
≥ Twice	16	7 (43.7)	09(56.25)	01(6.25)		
Total	184	47	67	04		

Table III. Isolation of *Brucella melitensis* from clinically suspected animals.

Sample type	No. of samples cultured	No. of isolates	Percentage (%)
Vaginal swab	05	01	20%
Placenta	02	0	0%
Milk	33	04	12.12%
Total	40	5	12.5%

Table IV. Antibiotic susceptibility of *B. melitensis* isolates from vaginal and milk samples of aborted animals.

Bacterial species	Antibiotic discs used	Inhibited zone diameter	Degree of susceptibility
B. melitensis	Cefoxitin	10mm	Partial sensitive
	Oxytetracycline	24mm	Highly sensitive
	Lincomycin	00mm	Resistant
	Erythromycin	00mm	Resistant
	Doxycycline	21mm	Highly sensitive
	Rifampicin	00mm	Resistant
	Cephalothin	00mm	Resistant
	Streptomycin	00mm	Resistant
	Bacitracin	00mm	Resistant
	Ciprofloxacin	21mm	Highly sensitive
	Gentamycin	00mm	Resistant

Antibiotic susceptibility profile of Brucella melitensis *isolated from milk swab samples*

B. melitensis species were identified based on microbiological and biochemical characteristics. The organism was subjected to a panel of eleven antimicrobials. The results indicating the efficacy of antibiotics against *Brucella melitensis* is presented in Table IV. The antimicrobial susceptibility test indicated that *B. melitensis* isolates was highly sensitive to oxytetracycline (24mm), doxycycline (21mm), ciprofloxacin (21mm) and partially sensitive to cefoxitin (10mm). While the organism found resistant to erythromycin, lincomycin, rifampicin, cephalothin, bacitracin, streptomycin and gentamycin.

DISCUSSION

This is the first study that was carried out in East part of Sindh (Dadu district) for investigation of Brucellosis, Chlamydiosis and Q fever prevalence. The objective to conduct this study was to collect essential information about the most commonly occurring etiologies of abortion in caprine and ovine in district Dadu of Sindh Province. Abortion in goats and sheep can be caused by several infectious agents. This study identified heavy burden of chlamydiosis in goats and sheep with high susceptibility in different areas of district Dadu Sindh. The present study indicated that among all three common abortifacient bacteria there was highest prevalence rate of Q fever after Chlamydiosis. This survey showed generally low prevalence of *Brucella melitensis* (Brucellosis) in the district Dadu, Sindh, Pakistan.

Q fever outbreaks in Netherlands related with sheep and goats have strengthen awareness of the disease as an important emerging zoonoses (Georgiev *et al*., 2013). Our study results exhibited that Q fever has high prevalence rate (25%) in the ovine and caprine population of study area. This finding is in accordance with studies of Zahid *et al*. (2016) who reported the highest prevalence of Q fever (30.8%) infection in goats and sheep of Punjab, Pakistan. While, Shabbir *et al*. (2016) reported 17.9% and 16.4% sero-prevalence of Q fever in goats and sheep, respectively. The literature reports only few studies on the prevalence of Q fever in country Pakistan (Kaplan and Bertagna, 1955; Ahmed, 1987; Zahid *et al*., 2016; Shabbir *et al*., 2016; Rashid *et al*., 2019; Memon *et al*., 2022). However, Kshash (2012) and Anastacio *et al*. (2013) have

reported higher prevalence in sheep than goats. In the current study 224 animals (n=112 goats and n=112 sheep) were investigated. Seroprevalence of Q fever determined in two species was higher in goats (33.6%) than in sheep (17.3%). This corelates with the results of Van den Brom *et al*. (2015) and Zahid *et al*. (2016). An overall prevalence of Q fever was 25.5%. These findings agreed with earlier studied Q fever seroprevalence in goats and sheep of Iran, Turkey and the Netherlands (Esmaeili *et al*., 2014; Arserim *et al*., 2011; Schimmer *et al*., 2011). Rahman *et al*. (2018) reported seroprevalence of 6.38% in domestic ruminants of Bangladesh. Rashid *et al*. (2019) have reported seroprevalence of 6.1% in cattle and buffaloes in institutional farms of Punjab, Pakistan.

Brucellosis is not only a disease of zoonotic importance but also it has economic and productive importance. The disease highly affects reproductive and productive potential of animals (Shabbir *et al*., 2013). In this study total seroprevalence of brucellosis in caprine and ovine was noted to be 2.17%. Our findings regarding occurrence of *Brucella* infections are close to the last reports (Shafee *et al*., 2016; Hussain *et al*., 2014; Iqbal *et al*., 2013), who have reported 2.3%, 7% and 10% prevalence in Baluchistan, the Punjab and Kohat, respectively. This study is in contrast to the reports of Gul *et al*. (2015) who have seen 1.91% and 2% prevalence in sheep and goats in Pakistan. In Punjab, Pakistan Shahzad (2017) has reported prevalence of 0.23% and 0% in goats and sheep, respectively. Shafy *et al*. (2016) and Rahman *et al*. (2011) reported sero-prevalence of 9.53% and 2.6% in Bangladesh. An overall seroprevalence of 1.72% of caprine brucellosis has been documented in India (Saikia *et al*., 2019). In Nepal, *B. melitensis* prevalence in caprine was found to be 2.6% (Pandeya *et al*., 2016).

In the current study, *Chlamydia abortus* was isolated from specimens belonging to goats and sheep with the history of recent abortion. There are reports of *Chlamydia* infection from small ruminants in the world (Ababneh *et al*., 2014; Berri *et al*., 2009). However, there is lack of studies on *Chlamydia* infection in sheep and goats in Pakistan. The first chlamydial isolation from birds have been reported in 2011 (Esmaeili *et al*., 2017). The studies of Esmaeili *et al*. (2017) corelate with our studies, who reported that 25% samples were positive for chlamydiosis. In the Middle Atlas and Northen Morocco Benkirane *et al*. (2015) conducted serological survey of 13 flocks of sheep and found all (100%) positive, while out of 10 flocks of goats 8 (80%) were seropositive. As indicated by the monetary significance of chlamydial abortion and its zoonotic implications. In the present investigation, detection of *Chlamydia* affirmed the ovine enzootic abortion disease in the examined groups.

Since there were no significant differences found neither in the overall abortion frequency among three abortifacient bacteria nor occurring among naïve, primiparous and multiparous goats and sheep. These results agree with the findings of Benkirane *et al*. (2015). Tekle (2016) have reported that parity and abortion frequency have no significant effect on the *Brucella* infection. Gul *et al*. (2015) in Punjab, Pakistan have noticed higher prevalence of brucellosis in multiparous animals (6-10 parity) as compared to nulliparous (naïve animals), statistically significant ($P< 0.004$) through Rose Bengal Plate Test, but non-significant through Indirect ELISA and all other serological tests. However, our findings are not in agreement with the finding of Hadush *et al*. (2013) who have observed effect of parity on prevalence of abortifacient bacterial pathogens. This difference might be due to managemental practices, rearing conditions and due to sampling methodology and timings. The present investigation was in accordance with the work of Kelkay *et al*. (2017) who have revealed that there was no significant effect of parity and pregnancy status on abortion.

The isolation of *Brucella melitensis* from the vaginal material and milk is also of great public health importance (Habtamu *et al*., 2015). In our study among the total isolates one isolate from vaginal swab and four isolates from milk were recovered. However, no isolates were detected from foetal membrane. This could be due to the slow growing nature of the bacterial pathogens and rapidly growing contaminants (Seleem *et al*., 2010). The current, 12.5% *B. melitensis* isolates from serologically positive animals with previous record of abortion is in correlation with preceding studies of *B. melitensis* (Cekovska *et al*., 2010). A researcher from Ethiopia has reported 13.84% *B. melitensis* isolates from milk and vaginal swab samples of aborted goats (Tekle, 2016). Among all isolates, 4 (12.12%) isolated *B. melitensis* from milk are parallel to the different previous findings, 3.2%, *Brucella* were isolated from cattle milk in Pakistan (Ali *et al*., 2014) and 4.4% in Turkey (Celebi and Otlu, 2011).

The bacterial species *B. melitensis* was susceptible to tetracycline, doxycycline and ciprofloxacin whereas resistant to gentamycin, erythromycin, streptomycin, cefoxitin, lincomycin, bacitracin, rifampicin and cephalothin. Almost identical results regarding the sensitivity of *B. melitensis* to oxytetracycline and other drugs were noted by Ilhan *et al*. (2013) who found *B. melitensis* highly sensitive to tetracycline.

CONCLUSION

It is concluded that the abortifacient bacteria *Chlamydia abortus, Coxiella burnetii* and *Brucella*

melitensis are prevailing in goats and sheep of district Dadu, Sindh. The present study confirms a considerably high prevalence of *C. abortus* and *C. burnetii* in sheep and goats. The parity and abortion frequency had no significant effect on the seropositivity of *Coxiella burnetii*, *Chlamydia abortus* and *Brucella melitensis*. *Brucella melitensis* was found resistant against the antibiotics erythromycin, lincomycin, rifampicin, cephalothin, bacitracin, streptomycin and gentamycin.

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IRB aproval

The study was approved by the IRB, Directorate of Advanced Studies, SAU, Tnadojam (No. DAS/2674 of 2019).

Statement of conflict of interest

The authors have declared no conflict of interest.

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