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Prevalence of *Coxiella burnetii* in Cattle and Buffaloes in the Islamabad Capital Territory and Other Selected Districts of Pakistan

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ABSTRACT

The present study was conducted to determine the sero-prevalence of *Coxiella burnetii* antibodies and associated risk factors in large ruminants (cattle and buffaloes) of four districts of Pakistan. A total of 175 milk samples were investigated from different areas of Pakistan. Out of 175 milk samples, species data for 28 samples were missing. The presence of *C. burnetii* antibodies was determined by indirect ELISA. The overall prevalence in large ruminants (cattle and buffaloes) was 6.1%. In cattle prevalence was higher (7%) than in buffaloes (5.3%). The difference in sero-prevalence between cattle and buffaloes was insignificant (X^2 =1.30, p=0.5, df=2). Based on the dairy animal breed, the stratification of *C. burnetii* antibodies showed that Mix breed had the highest sero-prevalence (7.7%). Bhimber had a high prevalence (40%), while ICT had no positive case. Mirpur and Rawalpindi showed prevalence rates of 8.9% and 5%, respectively. Statistical analysis (χ^2=10.61, df=10, p=0.3) indicated no significant differences between regions and *C. burnetii* antibodies. Associated risk factors for the seropositivity of *C. burnetii* antibodies were age of animals (X^2=1.20, df=2, p=0.5), contact with small ruminants (X^2 =.50, p=0 .7, df=2), absence of rodents (X^2=9.32 ,df=1, p=0.002), absence of ticks (X^2 = 1.989a, p=0.1, df=1), production system (X=.711[a] ,df=1, p=0.3), breeding method (X^2 =1.11 p=0.5, df=2), abortion history (X^2 = 1.31, p = 0.5, df = 2), health issue (X^2 = 1.61, p= 1.0, df=14),herd size (X^2=4.38 ,df=10,p=0.9), number of parities (X^2=0.88, df=2, p=0.6).The results of this study indicated that *C. burnetii* antibodies are prevalent in cattle and buffaloes in four districts (ICT, Rawalpindi, Bhimber Azad Kashmir, Mirpur Azad Kashmir) of Pakistan.

INTRODUCTION

"Q" fever was initially reported as an outbreak of febrile illness with an unknown origin that manifested as flu-like symptoms in slaughterhouse workers in 1935 (Sahu *et al*., 2024). Its host range is quite diverse, encompassing ticks, fish, reptiles, birds, ruminants, and humans (Shrestha *et al*., 2020).

The World Health Organization (WHO) lists Q fever, Rift Valley fever, and brucellosis as neglected zoonotic diseases (NZDs) that are prone to underreporting and incorrect diagnosis (Ibrahim *et al*., 2021). The *C. burnetii* was categorized as a category B biological agent by the US Centers for Disease Control and Prevention (CDC) because it can cause acute, debilitating diseases in specific human populations (Tagesu, 2019). Different animal species exhibit varying clinical presentations. In cattle, sporadic abortions, low birth weights, and infertility are more prevalent compared to sheep and goats. In sheep and goats, abortion typically occurs during the last trimester of pregnancy(Prajapati & Chauhan, 2024).The bacteria attach to phagocytes using Avb3 integrin and complement receptor CR3, destroying Phase II bacteria and allowing Phase I bacteria to survive in phagocytic cells (Roest *et al*., 2013). Immune responses to *C. burnetii* can last from a few months to several years, with the placenta's trophoblasts containing most metabolically active LCVs. The pregnant animals may experience abortion, stillbirth, weak offspring birth, and premature delivery (Ullah *et al*., 2022). Symptoms in humans include sudden headaches, high fever, chills, muscle soreness, respiratory tract infection, and severe headaches (España *et al*., 2020).

In Pakistan, information about Q fever and Coxiellosis is still lacking. There have only been twenty-four reports published since 1955 detailing the incidence of *C. burnetii* infection in both humans and animals in this country. According to these studies, the prevalence of Coxiellosis ranged from 4.6% to 40% in all livestock species and 10.2% to 26.8% in humans. Current findings involving

humans, camels, and small ruminants underscore the urgency of paying attention to this disease, particularly in regions where human-animal contact is still prevalent and milk supply chains are antiquated (Ullah *et al*., 2022). A study in seven districts of Punjab, Pakistan, found a seroprevalence of 23.66% in cattle and 27.23% in buffalo for *Coxiella burnetii*. Key risk factors included the absence of acaricide use, tick presence, recent abortions, and the presence of sheep and goats. (Hussain *et al*., 2022). Another study showed higher Q fever prevalence in goats (17.1%) than sheep (4.9%), with factors like species, age, and tick infestation influencing rates. (Amin *et al*., 2022). Research on dromedary camels with subclinical Coxiellosis revealed significant hematological and biochemical changes, emphasizing the need for further study. (Hussain *et al*., 2022). The discovery of *C. burnetii* DNA in Punjabi soils highlights the importance of the "One Health" approach in managing zoonotic diseases in Pakistan. Q fever is a zoonotic disease that primarily affects domesticated animals. Animals contract the infection through tick bites or inhaling the causative organism from a contaminated environment (Hadush *et al*., 2016).

In Pakistan, there have been calls to give priority to zoonotic diseases under the "One Health" paradigm (Ersoy). In this case, raising awareness among the public and the scientific communities in Pakistan and the surrounding nations will be beneficial. Although Q fever is a ubiquitous zoonotic disease, so far it has been neglected in Pakistan. The present study was planned to determine the sero-prevalence of Q fever and associated risk factors in cows and buffaloes in the four selected districts of Pakistan.

MATERIALS AND METHODS

Study Locales and Sample Size

This study was conducted at the Animal Health Section (AHS) Animal Sciences Institute, National Agriculture Research center (NARC), Islamabad and at the Department of Zoology Quaid-i-AzamUniversity, Islamabad. A total of 175 milk samples were collected from different districts of Pakistan including ICT, Rawalpindi, Mirpur Azad Kashmir and Bhimber Azad Kashmir.

Sample Collection

The milk samples were collected using 15ml falcon tubes, with 10ml collected in each tube. Post-collection, these samples were promptly placed in an icebox at 4°C to maintain their integrity during transportation to the laboratory. Upon reaching the laboratory, the milk samples underwent centrifugation at 3000 rpm for 3 minutes to obtain Lacto-sera. A structured questionnaire was used to record specific animal information during the sampling process. This information included species (cattle, buffalo), farm location (districts), age, breed, parity, abortion history, method of insemination, presence of rodents and small ruminants, tick presence, health issue, and general information of farm management. Parity of an animal means how many times an animal has given birth.

Serological Analysis

The indirect ELISA technique was employed for serological analysis for the detection of antibodies specific to C. burnetii in the milk samples. The ID Screen® Q Fever Indirect Multi- species ELISA is a systematic method for detecting anti- C. burnetii antibodies across various species. Samples and controls were placed in ELISA plate wells coated with C. burnetii antigens. Horseradish peroxidase (HRP) conjugate is added to the wells after excess materials are removed by washing. Together with the antibodies, this conjugate forms complexes. The wells are filled with Substrate Solution (TMB), which causes a blue coloring that corresponds to the quantity of antibodies present. Stop solution changes the blue color to yellow. The color intensity indicates the concentration of C. burnetii antibodies in the specimen. Through this procedure, anti- *C. burnetii* antibodies from a variety of species can be reliably detected.

Interpretation

S/P % for each sample was calculated using the following equation.

$$\frac{S}{P}\% = \frac{\text{OD sample} - \text{OD negative control}}{\text{OD positive control} - \text{OD negative control}} \text{ X } 100$$

The interpretation of SP values described by manufacturer is as follows: ≤40%=negative; ≥40% SP≤50%=doubtful; >50%=positive.

Statistical Analysis

IBM SPSS 27.0 for windows was employed for statistical analysis, and chi-square values were recoded.

RESULTS

ELISA based Sero-Prevalence of *Coxiella burnetii* Antibodies

A total of 175 milk samples of large ruminants (cattle and buffalo) were investigated for the sero-prevalence of *Coxiella burnetii* antibodies. The overall prevalence of *Coxiella burnetii* antibodies was 6.1%. The prevalence percentage in cattle recorded was 7 % and in buffalo it was lower than cattle 5.3%. The district-wise analysis revealed significant variations in prevalence rates. Bhimber exhibited the highest prevalence at 40%, indicating a potential hotspot for *C. burnetii* infection. In contrast, ICT reported no positive cases, suggesting a lower risk or absence of infection in that region. Mirpur had a prevalence of 8.9%, while Rawalpindi showed a prevalence of 5%. Sero-prevalence of *Coxiella burnetii* antibodies was the highest in age group 2 (6-8 years old). Stratification as per the herd size of animals was recorded highest in the herd size group (11-20 animals) 3.57% followed by the herd size group (21-30 animals) 3.13%.

Chi-square analysis was performed to find out the association between the different variables and prevalence of *Coxiella burnetii* antibodies. A total of 13 variables, viz., age, specie, abortion history, breeding method, contact with small ruminants and rodents, tick infestation, production system, geographical location, breed, herd size, parity, health status of an animal. Sero-prevalence of *Coxiella burnetii* antibodies was influenced by age, specie, geographical location and breeding method (Table 1).

Briefly, animals between 6 and 8 years of age were more likely to test positive as the age above 8 years and younger

than 6 years showed no positive case. The association between the age group and prevalence of *Coxiella burnetii* antibodies was found to be insignificant (*X*=1.20, df=2, p=0.5). Among 92 samples analyzed for parity (0-2, 3-5, above parity) showed only one positive case in "3-5" category. The chi-square analysis with (X=.887a, df=2, p=0.642) value showed that there was no statistically significant association between parity and *C. burnetii* antibody prevalence. The chi-square analysis (X^2 =1.610[a], p= 1.000, df=14) showed that there was no statistically significant association between health issues and *C. burnetii* antibody prevalence. Chi-square analysis for the overall relationship between the prevalence of *C. burnetii* antibodies and the history of abortions were non-significant (X2 = 1.317a, p = 0.518, df = 2). Moreover, the association between prevalence of *Coxiella burnetii* antibodies and tick infestation (X2 =1.989a, p=0.158, df=1), contact with small ruminants (X2 =.501a, p= .779, df=2), production system (X=.711a, df=1, p=.399), breeding method (X2 =1.112a p=.574, df=2), was found statistically insignificant.

Table 1

Chi-Square Analysis of the Q Fever Sero-Prevalence in Four Districts of Pakistan

Variable	Category	Pos. / Tested	*C. burnetii* Prev. %	Chi Sq(X^2)	P Value
District	Mirpur	4/45	8.9	27.661[a]	0.000
	Rawalpindi	1/20	5		
	Islamabad	0/72	Nill		
	Bhimber	4/10	40		
Age Groups	Above 8 years	0/20	Nill	1.204[a]	0.5
	5 to 8 years	1/42	2.4		
	3 to 5 years	0/30	Nill		
Species	Buffalo	4/76	5.3	1.300[a]	0.5
	Cattle	5/71	7		
Breed	Mix breed	1/16	6.3	10.225[a]	0.9
	Cross-bred	0/13	Nill		
	Neli Ravi	0/23	Nill		
	Australian	0/6	Nill		
	Australian Jersey	0/2	Nill		
	Cholistani	0/2	Nill		
	Desi	0/4	Nill		
	Friesian	0/3	Nill		
	Friesian Australian	0/1	Nill		
	Friesian Jersey	0/1	Nill		
	Jersey	0/7	Nill		
	Jersey Sahiwal	0/2	Nill		
	Sahiwal	0/3	Nill		
	Sahiwal Australian	0/4	Nill		
Production system	Closed	1/54	1.9	.711[a]	0.3
	Open	0/38	Nill		
Herd size (No of animals at farm)	Above 50	0/8	Nill	4.382[a]	0.9
	41 to 50	0/12	Nill		
	31 to 40	1/21	4.7		
	21 to 30	1/32	3.1		
	11 to 20	1/28	3.6		
	1 to 10	1/18	5.6		
Parities	Above 5	0/1	Nill	0.887[a]	0.6
	3 to 5 Parity	1/49	2		
	0 to 3 Parity	0/42	Nill		
Tick infestation	Yes	1/62	1.6	1.989[a]	0.1
	No	0/30	Nill		
Breeding	AI	4/74	5.4	1.112[a]	0.5
	Natural	5/73	6.8		
Small ruminant contact	Yes	6/105	5.7	.501[a]	0.7
	No	3/42	7.1		
Contact with rodents	Yes	0/83	Nill	9.324[a]	0.002
	No	1/9	11.11		
Health issue	No	12/157	7.6	1.610[a]	1.0
	Actinomyces	0/2	Nill		
	Fasciolosis	0/1	Nill		
	HS	0/2	Nill		
	Mastitis	0/6	Nill		
Abortion	Yes	0/15	Nill	1.317[a]	0.5
	No	12/160	7.5		

DISCUSSION

Coxiellosis, a disease known since 1935, gained attention after a human outbreak in the Netherlands between 2007 and 2010 (Georgiev *et al.*, 2013). It poses a significant threat to Pakistan, where livestock is crucial for the rural economy, supporting 8 million families and accounting for over 35% of their income (Arif, 2018). The intimate relationship between people and animals and farmers' lack of knowledge highlights the risk of zoonotic disease transmission. This study investigated the prevalence of *C. burnetii* antibodies in four selected regions of Pakistan.

This study showed the highest prevalence in a specific

district Bhimber (40%). The significant variability in prevalence among districts emphasizes the importance of local factors influencing the spread of *C. burnetii*. This suggested a geographical diversity in the prevalence of *C. burnetii*, emphasizing the need for region-specific surveillance and control measures. Results of present study are in agreement with the studies in Pakistan as both studies investigated the highest prevalence in cattle, study in Punjab province under the administered control of Livestock and Dairy Development (L&DD) Department, Government of the Punjab and University of Agriculture reported highest prevalence in cattle (7.63%) and 4.4% in buffalos (Rashid *et al*., 2019). Similarly, according to Shujat *et al*., 2023 highest prevalence Was recorded in cattle.

The observed prevalence in this study aligns with similar investigations conducted in India and other countries, supporting the idea that *C. burnetii* is more prevalent in cattle milk compared to other animals. The prolonged shedding of the bacterium in cattle milk, as opposed to shorter vaginal shedding, underscores the potential role of cattle milk in Q fever epidemiology and its impact on public health. These findings resonate with a study from Punjab India, which reported an overall prevalence of 8.7% in cattle (Keshavamurthy *et al*., 2019). In this study results indicated no statistically significant associations between the *C. burnetii* antibodies and breed of an animal and herd size of cattle and buffaloes. On the other hand, Coxiellosis prevalence according to cattle breed demonstrated that Cholistani breed had the highest prevalence (13%) followed by Sahiwal (7.5%) and crossbreed (2.6%). (Rashid *et al*., 2019). In contrast, study in Northern Ireland demonstrated that there is statistically significant association between Q fever and Friesian breed and odds of *C. burnetii* infections increases with the increase in herd size (McCAUGHEY *et al*., 2010). This study has some limitations, including the lack of complete data for all samples. Future research should aim to fill these gaps, considering the potential influence of diverse factors such as climate, farming practices, and animal interactions. Public health is addressing the prevalence of Coxiellosis in animal populations, as human infections can lead to serious health problems and economic losses. A One Health approach is crucial for monitoring and controlling Coxiellosis, involving coordination between veterinary and public health organizations. Preventive measures like animal immunization programs and awareness campaigns can reduce the spread of C. burnetii, while ongoing research is essential for designing effective control strategies.

These findings underscore the need for more extensive surveillance and epidemiological studies to better understand the factors influencing the spread of *C. burnetii* in different regions. The high prevalence in specific districts, such as Bhimber, suggests that targeted interventions and enhanced biosecurity measures are necessary to control and prevent the spread of Q fever in these areas.

CONCLUSION

In current study prevalence of Coxiellosis in cattle and buffaloes in ICT and some other selected districts of Pakistan were studied. Highest prevalence was observed in Bhimber, followed by Mirpur, Azad Kashmir. The various factors that were found to be associated with the prevalence of Coxiellosis were age, breed, health status, method of insemination, small ruminants and rodents' presence on the farms, herd size, parity, abortion history, tick infestation. However, since this study involved a small number of samples, future studies are needed to gain a better understanding of the prevalent rate of this disease as well as the risk factors potentially associated with the occurrence of this disease. The prevalence of *C. burnetii* pathogen causes zoonotic disease Coxiellosis in dairy animals of Pakistan raises concerns regarding the possibility of transmission to the local human population.

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