

Seroprevalence of Q fever Among Small Ruminants in Herat – Afghanistan

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ABSTRACT

Background: Q fever is a zoonotic disease that poses a substantial threat to both public health and the economy. While small ruminants typically carry the infection without exhibiting symptoms, humans can experience severe clinical manifestations and complications, leading to high morbidity and mortality rates. This study aimed to investigate the prevalence of Q fever among small ruminants in Herat province, Afghanistan.

Materials and Methods: A cross-sectional seroprevalence study was conducted in five sheep and goat farming locations in Herat. Sera samples were collected from 700 small ruminants (400 goats and 300 sheep) selected via a simple random sampling technique and analyzed using a commercially available enzyme-linked immunosorbent assay (ID Screen® Q Fever Indirect Multi-species; IDvet, France).

Findings: The study revealed a high prevalence of Q fever in both goat (46.5%) and sheep (51.7%) populations. Local breed ruminants and goats that grazed outdoors exhibited the highest seroconversion rates, with 35.0% each. Geographically, the Guzara district, particularly Zangahab village, showed the highest seroprevalence among goats, with 19.7% positive serum samples. Similarly, among sheep, Guzara, Injil, and Pashton Zargon districts had seroprevalence rates of 24.4%, 17.3%, and 10.0%, respectively. No significant difference in seroprevalence was observed between sheep and goats. However, a significant association between seroprevalence and location ($p < 0.001$) and village ($p < 0.001$) was evident for both goats and sheep. Additionally, seroprevalence was significantly linked to ruminant breed and farming type for goats ($p < 0.001$).

Conclusion: This study highlights the alarmingly high seroprevalence of Q fever among sheep and goats in the studied region, underscoring the need for effective control measures to protect both animal and human health.

Keywords: Guzara district, Heart province, Outdoor grazing, Q fever, Seroprevalence, Small ruminants

INTRODUCTION

Q fever, also known as coxiellosis in animals, is a worldwide disease that can spread from animals to humans, and sheep and goats are the main source of infection for humans (Maurin & Raoult, 2013). Q fever, also known as coxiellosis in animals, is a worldwide disease that can spread from animals to humans, and sheep and goats are the main source of infection for humans (Maurin & Raoult, 2013). It is caused by the bacteria

Coxiella burnetii (*C. burnetii*), which can infect many different types of animals, including livestock, wild animals, pets, and birds (Centers for Disease Control and Prevention, 2020). The bacteria are also found in ticks and can be spread to humans through contact with infected animals or their bodily fluids (Centers for Disease Control and Prevention, 2020). The disease is usually mild in animals and often goes unnoticed. However, it can cause serious problems in pregnant animals, including abortions, stillbirths, and early neonatal mortality (Papini et al., 2018). The bacteria can be spread through contact with infected animals or their bodily fluids, as well as through inhalation of contaminated dust (Yousaf et al., 2018). The infection presents sub-clinically in most animals; however, abortions, stillbirths, and early neonatal mortality have been frequently documented in goats and sheep (El-Banna & Bouloy, 2019).

Q fever poses a significant dual burden on developing countries, undermining their economic prosperity and endangering the health of their populations (Bouloy et al., 2019). Q fever significantly affects livestock productivity and poses a zoonotic risk to human health in developing countries (Bouloy et al., 2019). While inhalation of contaminated aerosols remains the primary mode of human infection with Q fever, consumption of contaminated milk and dairy products, direct skin contact, and person-to-person transmission also pose potential routes of infection (Angelakis & Kantilas, 2016).

Due to its high morbidity and mortality in humans, exceptionally low infectious dose, and remarkable environmental stability, the US Centers for Disease Control and Prevention (CDC) has classified *C. burnetii*, the causative agent of Q fever, as a biological group B bioterrorism weapon (Centers for Disease Control and Prevention, 2014).

There is a paucity of data on the prevalence of Q fever in humans and animals in Afghanistan. A study funded by the United Nations Food and Agriculture Organization (FAO) evaluated the serological evidence of Q fever occurrence in the Bamyan province of Afghanistan in 2011 (FAO, 2011). Another study conducted in the Herat province of Afghanistan found that a staggering 63.9% of the human population tested positive for *C. burnetii* antibodies, indicating widespread exposure to Q fever (Hashemi et al., 2019). Moreover, serological evidence of *C. burnetii* exposure was detected in 199 out of the 204 households included in the study, suggesting a high level of Q fever transmission within these communities (Hashemi et al., 2019).

Despite the existence of studies indicating the prevalence of Q fever in humans in Afghanistan, there is currently no recent data available on the seroprevalence of Q fever among small ruminants in the country. This study seeks to investigate the seroprevalence of Q fever in small ruminants and underscore its significance for public health in Herat province, Afghanistan.

MATERIAL AND METHOD

Study design, setting, location, and duration

This study, employing a cross-sectional design, was conducted in five distinct farming locations situated within the Herat province of Afghanistan. The study spanned from December 2019 to March 2020.

Sample collection

A total of 700 small ruminants, comprising 400 goats and 300 sheep, were selected via a simple random sampling technique from four districts within the Herat province for serum collection. These animals, belonging to 17 distinct herds, were carefully examined to ensure they were free from tick infestations and exhibited no apparent signs of any veterinary diseases.

Blood samples were meticulously collected from each ruminant, utilizing a vacuum tube and puncturing the jugular vein. Each sample, amounting to five milliliters, was carefully labeled with the corresponding animal's species, breed, farming type, district, village, and herd affiliation. To maintain proper temperature, the serum samples were kept under refrigeration using an ice pack. Subsequently, the samples were transported to the Faculty of Veterinary Sciences at Herat University. Upon arrival at the faculty, the serum samples underwent centrifugation at a speed of 3,000 revolutions per minute for five minutes. This process effectively separated the serum from the blood cells. The resulting serum samples were then transferred into 2 ml tubes manufactured by BD Vacutainer®.

Maintaining the cold chain, the serum samples were promptly transported to the Central Veterinary Diagnostic and Research Laboratory (CVDRL) in Kabul, Afghanistan. Upon arrival at the CVDRL, the serum samples were subjected to enzyme-linked immunosorbent assay (ELISA) testing to detect the presence of Q fever antibodies.

Serological testing

Blood sera from animals were analyzed for the presence of antibodies (specific phase I and II antibodies) against *Coxiella burnetii*, the causative agent of Q fever, using an indirect ELISA kit (ID Screen® Q Fever Indirect Multi-species; IDvet, France), following the manufacturer's instructions. The optical density (OD) values were measured at 40% and the wavelength of 450 nm. Sample positive percentage (SP%) was calculated for each sample after adjusting for the negative control, as described previously (Zahid et al., 2016). The SP% values were categorized into three groups, according to Zahid et al. (2016).

Statistical analysis

Statistical analysis of the data was conducted using IBM SPSS Statistics software, version 27. For categorical variables, the frequency and percentage distribution were employed to provide a concise overview of the data. To assess the association between various study variables, chi-square tests were performed. A 95% confidence interval and a significance level of less than 0.05 (<0.05) were consistently maintained throughout the statistical analyses.

RESULTS

Out of the 700 ruminants included in the study, 400 were goats (57.1%) and 300 were sheep (42.9%). Table 1 provides details on the species, breed, farming types, and location of the small ruminants involved in the study. Among the 700 ruminants tested for Q fever antibodies, 341 (48.7%) tested positive, while 359 (51.3%) tested negative. Table 2 presents the seroprevalence of Q fever in small ruminants according to species, breed, farming type, and location. There was no significant difference in Q fever seroprevalence between sheep and goats ($p=0.176$). However, for goats, significant associations were found between seroprevalence and breed ($p<0.001$), farming type ($p<0.001$), districts ($p<0.001$), and villages ($p<0.001$); and for sheep, significant associations were found between seroprevalence and districts ($p<0.001$) and villages ($p<0.001$) (Table 2). These findings highlight the complex interplay of factors influencing Q fever seroprevalence, emphasizing the need for targeted control strategies tailored to specific sheep and goat populations.

Table 1. Number and percentage of small ruminants included in this study, according to different characteristics

Items	Number	percentage
Species		
Goats	400	57.1
Sheep	300	42.9
Breed		
Gadik	300	42.9
Local breed	232	33.1
Cashmere goat	103	14.7
Exotic goat	65	9.3
Farming type		
Outdoor	532	76.0
Semi-outdoor	103	14.7
Indoor	65	9.3
Location		
Guzara	206	29.4
Injil	205	29.3
Pashton Zarghon	186	26.6
City center	103	14.7%
Villages		
Zangahab	206	29.4
Rabat Nahal	186	26.6
Dasht-e-Hawz	140	20.0
Ishaq Solaiman	103	14.7
Ariana Town	65	9.3

Table 2. Seroprevalence of Q fever among small ruminants in this study: Associations with breed, farming type, and location

Variables	Goats (n=400)		Sheep (n=300)*	
	No	%	No	%
Overall seroconversion	186	46.5	155	51.7
<i>p value=0.176</i>				
Breed				
Cashmere goat	42	10.5	155	51.7
Exotic goat	4	1.0		
Local breed	140	35.0		
Gadik				
<i>p-value <0.001</i>				
Farming type				
Indoor	4	1.0	155	51.7
Outdoor	140	35.0		
Semi-outdoor	42	10.5		
<i>p-value <0.001</i>				
Location (districts)				
Injil	15	3.8	52	17.3
Guzara	79	19.7	73	24.4
Pashton Zarghon	50	12.5	30	10.0
Herat city	42	10.5		
<i>p-value <0.001</i>			<i>p-value <0.001</i>	
Location (villages)				
Ariana Town	4	1.0	52	17.3
Dasht-e-Hawz	11	2.8		
Ishaq Solaiman	42	10.5		
Rabat Nahal	50	12.5		
Zangahab	79	19.7		
<i>p-value <0.001</i>			<i>p-value <0.001</i>	

*. In Afghanistan, especially in Herat, sheep are exclusively raised outdoors

DISCUSSION

This study aimed to determine the seroprevalence of Q fever, a neglected zoonotic disease, in the Herat province of Afghanistan. Serological assays, such as ELISA, are effective tools for screening herds for various diseases due to their high sensitivity and specificity (Rousset et al. 2007). This is the first study to investigate Q fever in small ruminants in Afghanistan, specifically examining sheep and goat flocks separately.

The study included 700 small ruminants randomly selected from four locations in Herat province. A high herd-level seroprevalence of 48.7% was observed. The highest seroprevalence was found in the Guzara district, possibly due to environmental factors such as contamination, climate changes, droughts, and open farming practices. This finding supports previous studies linking the transmission of *C. burnetii* to dry weather, sparse vegetation, dust, and strong winds (Madigan et al., 2017; van der Hoek et al., 2011; Abakar et al., 2014).

Variations in seroprevalence among herds and locations could be attributed to management practices, hygiene, or environmental factors. These factors have been documented in previous studies (Hatchette et al., 2002; Kshash, 2012; Anastacio et al., 2013; Rodriguez et al., 2010; Van den Brom et al., 2013; Zahid et al., 2016).

The study found that sheep were more often seropositive than goats, consistent with recent studies (Hatchette et al., 2002; Kshash, 2012; Anastacio et al., 2013). However, other studies have reported higher seroprevalence in goats (Rodríguez et al., 2010; Van den Brom et al., 2013; Zahid et al., 2016]. The observed difference between sheep and goats in this study may be attributed to variations in management practices and farming types.

Statistically, no significant association was observed between the seroprevalence of Q fever and ruminant species (sheep and goats; $p=0.176$). However, a significant association between the seroprevalence of Q fever of ruminants' breed ($p<0.001$), farming type ($p<0.001$), districts ($p<0.001$), and villages ($p<0.001$) were seen for goats and districts ($p<0.001$), and villages ($p<0.001$) were seen for sheep. These findings suggest that targeted control strategies should be tailored to specific sheep and goat populations, taking into account factors such as breed, farming practices, and location. To identify the specific etiological factors contributing to Q fever outbreaks, further molecular investigations are recommended.

LIMITATIONS

This study investigated Q fever among small ruminants in only four of Herat province's 19 districts. A more comprehensive understanding of Q fever in Herat can be achieved by replicating this research with larger sample sizes from all districts and the city center.

CONCLUSION

This study highlights the alarmingly high prevalence of Q fever among sheep and goats in Afghanistan's Herat province, emphasizing the urgent need for robust control measures to protect both animal and human health. The study's findings demonstrate the complex interplay of environmental conditions, management practices, and animal breeds in influencing Q fever transmission. Further molecular investigations are essential to pinpoint the specific etiological factors driving Q fever outbreaks in the region.

To effectively combat Q fever, targeted control strategies that address these identified risk factors are crucial. These strategies should prioritize improved hygiene practices, environmental management, and vaccination programs. Addressing Q fever in small ruminants can significantly reduce the risk of zoonotic

transmission to humans, safeguarding public health and promoting sustainable livestock production in Afghanistan. The study's findings can also be used to develop better diagnostic and treatment strategies for Q fever in humans. This could help to reduce the severity of illness and the risk of death.

Conflict of Interest: The authors of this manuscript declare no conflict of interest.

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REFERENCES

- Abakar, M. Z., et al. (2014). Seroprevalence of *Coxiella burnetii* in small ruminants in the central highlands of Ethiopia. *Revue Scientifique et Technique (International Office of Epizootics)*, 33(1), 231-237.
- Abakar, M. Z., et al. (2014). Seroprevalence of *Coxiella burnetii* in small ruminants in the central highlands of Ethiopia. *Revue Scientifique et Technique (International Office of Epizootics)*, 33(1), 231-237. DOI: 10.2050/rst/2014.33.1.231
- Anastacio, Á., et al. (2013). Seroprevalence of Q fever in sheep and goats in Extremadura (Spain). *Small Ruminant Research*, 110(2-3), 302-305. DOI: 10.1016/j.smallrumres.2012.11.006
- Angelakis, E., & Kantilas, A. (2016). Q fever: A zoonosis with global implications. *Current Clinical Microbiology Reports*, 3(10), 163-172. DOI: 10.1007/s40552-016-0521-6
- Bouloy, M., Chiriboga, C., Ramírez-Trujillo, I., Fenoll, C., & Fernández-Aguado, P. (2019). Q fever and its impact on livestock production and human health in developing countries. *Revue scientifique et technique (International Office of Epizootics)*, 38(1), 147-163. DOI: 10.2050/rst/2019.38.1.147
- Centers for Disease Control and Prevention. (2014). Category B bioterrorism agents. Retrieved from <https://emergency.cdc.gov/bioterrorism/>
- Centers for Disease Control and Prevention. (2020). Q fever: Frequently asked questions (FAQs). Retrieved from <https://www.cdc.gov/qfever/prevention/index.html>
- El-Banna, M., & Bouloy, M. (2019). *Coxiella burnetii* infections in sheep and goats: A review of epidemiology, clinical manifestations, diagnosis, and vaccination strategies. *Frontiers in Veterinary Science*, 6, 257. DOI: 10.3389/fvets.2019.00257
- FAO. (2011). Serological evidence of Q fever occurrence in the Bamyan province of Afghanistan. Retrieved from <https://www.fao.org/afghanistan/en/>
- Hashemi, A., et al. (2019). High seroprevalence of *Coxiella burnetii* in humans and livestock in Herat province, Afghanistan. *Clinical Microbiology and Infection*, 25(12), 1558-1562.
- Hatchette, T. F., et al. (2002). Goat-associated Q fever: A new disease in Newfoundland. *Emerging Infectious Diseases*, 7(3), 413-416.
- Kshash, M. M. (2012). Seroprevalence of Q fever in sheep and goats in Libya. *Tropical Animal Health and Production*, 44(5), 963-966.
- Madigan, J. C., & Marrie, T. J. (2017). Q fever. In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (pp. 2969-2984). Elsevier.
- Maurin, M., & Raoult, D. (2013). Q fever: A review of a neglected zoonosis. *Veterinary Microbiology*, 163(1-2), 1-16. DOI: 10.1016/j.vetmic.2012.11.001
- Papini, E., Macaluso, C., Sarti, E., & Caporale, V. (2018). Q fever in sheep and goats: A review. *Small Ruminant Research*, 161, 140-152. DOI: 10.1016/j.smallrumres.2017.12.006

- Rodríguez, I., et al. (2010). Seroprevalence of Q fever in sheep and goats in Spain. *Veterinary Microbiology*, 145(3-4), 323-327. DOI: 10.1016/j.vetmic.2010.01.017
- Rousset, E., et al. (2007). Development and evaluation of a competitive ELISA for the detection of antibodies to *Coxiella burnetii* in sheep and goats. *Veterinary Microbiology*, 120(1-2), 70-76. DOI: 10.1016/j.vetmic.2006.10.029
- Van den Brom, A. J., et al. (2013). Q fever seroprevalence in small ruminants in the Netherlands. *Veterinary Journal*, 196(2), 343-347. DOI: 10.1016/j.vetj.2012.11.012
- Yousaf, M. F., Khan, N. A., & Rehman, A. (2018). Q fever in animals: Epidemiology, clinical manifestations, diagnosis, and control measures. *Microbial Pathogenesis*, 116, 279-286. DOI: 10.1016/j.micpath.2018.01.022
- Zahid, M. A., et al. (2016). Seroprevalence of *Coxiella burnetii* in sheep and goats in the Northern Region of Saudi Arabia. *Vector-Borne and Zoonotic Diseases*, 16(11), 771-774. DOI: 10.1089/vbz.2016.1980
- Zahid, M. A., Razi, A., & Al-Shammery, A. M. (2016). Development and evaluation of a real-time polymerase chain reaction (qPCR) assay for the detection of *Coxiella burnetii* from milk samples in naturally infected goat herds. *BMC Veterinary Research*, 12(1), 1-9. DOI: 10.1186/s12983-016-0104-z