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ORIGINAL ARTICLE

Molecular assessment of *Coxiella burnetii* in horses in Northwestern Iran

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ABSTRACT

Background and Objectives: Q fever is a frequently occurring illness that is induced by the bacterium *Coxiella burnetii (C. burnetii*) that can infect humans and various animals. It targets the macrophage cells in the tissues, and circulating monocytes.
Materials and Methods: This study was conducted between 2022 and 2023 in the West Azerbaijan and Ardabil provinces of northwestern Iran to examine the presence infection of *C. burnetii*. Specimens were obtained by swabbing from 140 mares (70 from each province) and 20 stallions (10 from each province) which were apparently healthy, and their DNA was analyzed using quantitative PCR assay detecting the *IS1111* element of the bacterium.
Results: The findings indicated that a mere 0.625% of the examined specimens tested positive for *C. burnetii*. Among the entire set of specimens, a single female horse from the region of Ardabil was found to be the carrier of the bacterium.
Conclusion: This suggested that even though horses may not display any clinical symptoms, they can still harbor *C. burnetii* and contribute to its transmission. Therefore, the potential contribution of horses to Q fever transmission should be considered.

Keywords: *Coxiella burnetii*; Horse; Iran; Polymerase chain reaction

INTRODUCTION

Q fever is a common illness in developing countries including Iran. It is the result of infection by a bacterium that obligately reproduces within host cells known as *Coxiella burnetii*. This bacterium is gram-negative and can infect humans and various animals (1). *C. burnetii* is found in a diverse range of hosts, including domestic and wild animals, pets, birds, fish, reptiles, and even invertebrates like ticks, across the world, with New Zealand being the only known exception (2). While goats, cows and sheep have been introduced as the primary sources of *C. burnetii*, the impacts of this organism on horses appears to have been overlooked. For instance, this bacterium has been cited as one of the causes of abortions in French horses.

The identification of positive cases of *C. burnetii* infection in equines presents a significant opportunity to recognize these animals as potential reservoirs of the pathogen, thereby enhancing awareness regarding the risk of transmission of this infection to both humans and other animal species. Behaviors such as touching horses and ponies, as well as activities such as continuous and long-term horseback riding are among the potential risk factors that can

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individually cause the spread of Q fever from horses to humans. Engagement in equine management practices, such as stable sanitation, hay manipulation, and mane maintenance, may lead to the inhalation of aerosolized particles harboring *C. burnetii*, posing a risk of human infection through respiratory exposure (3).

In terms of epidemiology, Q fever is a disease endemic to Iran, and therefore the interest in research and diagnosis of the disease in this country has been substantial. Nokhodian et al. performed a systematic search of articles published from January 2000 to 2015 to evaluate the occurrence and distribution of *C. burnetii* infections in both human and animal populations. Their results indicated an overall prevalence of antibodies for Q fever among animals of 27.00% (4).

In spite of this, the role of horses in the epidemiology of *C. burnetii* as a potential reservoir has not yet been definitively established (5). Previous studies on *C. burnetii* focused more on humans and ruminants. The role of horses as hosts in Iran was investigated by Jaferi et al. (6). In northern Iran (Golestan province) 7.50% of horses tested positive for *C. burnetii*. This suggests that horses in this region are significant from an epidemiological and health protection perspective concerning Q fever (7).

The diagnosis of infection with *C. burnetii* varies according to the sample type and the specific purpose of the testing (8). Hence, several diagnostic methods have been developed based on polymerase chain reaction (PCR) to analyze the DNA of *C. burnetii* in clinical samples. A transposon mutagenesis screen has revealed 512 essential genes in *C. burnetii*. The selection of the *IS1111* gene for this study is based on its high copy number in *C. burnetii*, which enhances the sensitivity of molecular detection methods and underscores its relevance as a reliable target for identifying this pathogen. These essential genes are involved in critical metabolic pathways such as the mevalonate pathway, peptidoglycan synthesis, and biotin synthesis. Some of the identified essential genes are typically associated with *C. burnetii* virulence, such as predicted type IV secretion system effector genes, suggesting these virulence factors also play crucial roles in the basic biology of the pathogen (9-11). Techniques based on high copy number genes such as *IS1111* element sequences which have a high sensitivity can also be considered (8, 12). According to prior research, PCR is recognized as a reliable laboratory method for analyzing *C. burnetii*. In particular, the trans-PCR approach, which targets transposon-like elements, proves effective in detecting *C. burnetii* through the application of *IS1111* primers. The genome contains a minimum of 56 instances of integrated genetic elements, which guarantees a high degree of detection sensitivity (13). The trans-PCR method enables the identification of *C. burnetii* in samples such as genital swabs, milk, and feces within a timeframe of fewer than six hours.

The probability of zoonotic transmission of *Coxiella* organisms to human hosts increases subsequent to contact with equines harboring the pathogen, especially during equestrian activities and equine management practices. Hence, since the trading of horses has recently become more popular in Iran, this investigation aimed to quantify the prevalence of *C. burnetii* in in swab samples from the clitoris, clitoral fossa, and urethral processes of mares and the penile skin or urethral processes of stallions using quantitative PCR in Iran's northwestern regions, specifically the provinces of Ardabil and West Azerbaijan.

MATERIALS AND METHODS

Determination of sample size and collection of specimens. In this study, the sample size was calculated based on an anticipated disease prevalence of 10.00%, a permissible margin of error of 5.00%, and a 95.00% confidence level, employing a simple random sampling approach. Based on these criteria, a minimum of 139 samples was deemed necessary. For this study, 160 horses were randomly chosen from various regions across the provinces of West Azerbaijan and Ardabil during the period spanning 2022 to 2023 (Fig. 1). Sampling was done across all seasons. Swab samples were taken from the clitoris, clitoral fossa and the urethral process of 70 mares and the penile skin or the urethral process of 10 stallions (140 mares and 20 stallions in total from the two provinces). All this information about mares and stallions was recorded in the acquisition history table (data not shown). The age of the horses was assessed based on the appearance of teeth. All the farms from which the horse samples were obtained had conventional stables.

DNA extraction. The swabs prepared for the PCR test were immersed in a microtube supplemented with 500 µL of PBS. Then, the collected samples

Fig. 1. Map of northwestern Iran highlighting the study areas.

were coded and recorded in a questionnaire and placed in an ice-cooled container for transportation to the Microbiology Laboratory at the University of Tabriz's Faculty of Veterinary Medicine. A 10-minute centrifugation process at 3,000 rpm was applied to the microcentrifuge tubes. Genomic DNA was isolated from the swab specimens utilizing a commercially available nucleic acid purification system (FavorPrep™ Tissue Genomic DNA Extraction Kit, Favorgen Biotech Corp., Taiwan) in accordance with the manufacturer's standardized protocol.

Quantitative molecular identification of *C. burnetii*. Quantitative PCR analysis targeting the *IS1111* insertion sequence of *C. burnetii* was conducted at the Pasteur Institute of Iran, employing specific oligonucleotide primers and fluorescent probes (14). The quantitative PCR amplification was performed in a total reaction volume of 20.00 µL, comprising the following components: 10.00 µL of 2X Real Q Plus Master Mix for Probe (Amplicon, Odense, Denmark), forward and reverse oligonucleotide primers at a final concentration of 900 nM each (5'-AAAACGGATAAAAAGAGTCTGTGGTT-3' and 5'-CCACACAAGCGCGATTCAT-3', respectively), a fluorescent probe at 200 nM (5'-6-FAM-AAAGCACTCATTGAGCGCCGCG-TAMRA-3'), 4.00 µL of the extracted genomic DNA template, and 5.00 µL of nuclease-free water to achieve the final volume (14). The amplification process was conducted utilizing a Corbett 6000 Rotor-Gene System (Corbett, Melbourne, Australia). The quantitative PCR thermal cycling protocol comprised an initial denaturation phase at 95.00°C for 10 minutes, succeeded by 45 cycles of denaturation at 94.00°C for 15 seconds and combined annealing/extension at 60.00°C for 60 seconds. Nuclease-free water and genomic DNA extracted from the Nine Mile strain (RSA 493) were employed as negative and positive controls, respectively. Data analysis was performed using Rotor-Gene® Q 2.3.5 software (Qiagen, Germany), with samples exhibiting cycle threshold (Ct) values ≤40 considered positive for the target sequence (14).

RESULTS

The horses participating in the study had no previous history of disease. The age of both stallions and mares varied between 2 and 17 years (Table 1). The range of foaling events varied from 0 to 12, with the majority of horses experiencing between 1 and 3 foalings. Additionally, the number of unsuccessful pregnancies recorded varied from 1 to 10, while temporary infertility varied between 1 month and 4 years.

Real-time PCR. Findings from the analysis revealed that out of 160 samples taken from mares and stallions, only one sample (0.625%) was positive in the province of Ardabil. This seven-year-old mare had foaled twice, with an unsuccessful pregnancy and infertility for 4 months. *Coxiella burnetii* remained undetected across all tested samples obtained from the stallions in the Ardabil province, similar to the mares and stallions from the West Azerbaijan province.

DISCUSSION

In recent years, the extensive spread of Q fever throughout different regions including Iran and other Middle Eastern countries has raised public health

Table 1. Risk factors influencing *C. burnetii* acquisition in horses

Risk factors	Category	Total No.
Age	< 5	53
	5 - 10	87
	> 10	20
Gender	Female	140
	Male	20
Breed	Arab	2
	Kurdish	2
	Crossbred	156

concerns worldwide in regards to infections among humans and animals (15, 16). The serological incidence of *C. burnetii* infection in human and animal populations between 1982 and 2010 was 2.00 and 10.00% in four European countries (17). Epidemiological investigations conducted by Esmaeili et al. and Ghasemian et al. revealed that the prevalence of acute Q fever among patients presenting with compatible symptomatology in northern and northwestern Iran was 13.80% and 5.37%, respectively (18, 19). Moradnejad et al. reported a prevalence of 30.77% infectious Q fever in cases of endocarditis with negative blood cultures (20). In addition, there are numerous reports of *C. burnetii* epidemiology in domestic livestock and wild fauna, as well as in ticks, milk, and dairy products from Iran (15, 21, 22).

Consequently, considering the importance of this disease in medicine and veterinary medicine, in this research, the prevalence of *C. burnetii* was investigated in the horse farms of West Azerbaijan and Ardabil provinces. Due to the low sensitivity and specificity of serological tests (23), the real-time PCR method was employed in this study for the detection and identification of *C. burnetii*.

In general, the seroprevalence of Q fever in equins is not markedly different from the average seroprevalence in ruminants based on published studies. For instance, seroprevalence rates in European countries were reported to be 15.80% in horses, 15.00-21.00% in cattle, 2.50-88.00% in goats, and 3.50-56.00% in sheep (8). Conversely, blood serum collected in the initial phase of systemic illness serves as an appropriate sample for the genomic analysis of pathogen, as the pathogen's genome may be present. However, conventional PCR is not ideal for diagnosis because of the inhibitors present in blood and the low concentration of microbial nucleic acids in serum (24). In addition, serologic methods can detect active and previous antibodies, whereas PCR is limited to identifying the acute stage of infection in serum samples (23). Hence, real-time PCR, which is a much more sensitive method than conventional PCR, can help identify *C. burnetii*. Since this disease is transmitted in animals via the placenta, urine, feces, and body fluids, all these can be utilised to take samples from for the initial identification.

Based on the above, it is difficult to compare the present study with previous studies carried out in horses with the purpose of evaluation *C. burnetii*, as the type of sample and the working methods were different in terms of specificity and sensitivity. According to the results of our study, out of 160 samples collected from mares and stallions in West Azerbaijan and Ardabil provinces, only one sample (0.625%) was positive which was in contrast to the results of studies conducted in the northwestern region and other parts of Iran and the world.

In the study conducted by Tehrani and Ownagh on the genomic detection of *C. burnetii* in horses in northwestern Iran, the results showed that out of 320 horse blood samples, the *IS1111* sequence was detected in 26 horses (13.8 percent), indicating the incidence of *C. burnetii* in horses in West Azerbaijan province (8). A molecular investigation conducted by Khademi et al. in the northern region of Iran (Golestan province) revealed that 7.50% of equine serum samples tested positive for *C. burnetii* DNA (7). In a study by Jaferi et al., the incidence of *C. burnetii* from vaginal swabs of horses in the northeast and southeast of Iran employing quantitative PCR was less than 8% (6). Seo et al. demonstrated that 1.30 percent of horse blood specimens exhibited positivity for *Coxiella*-like organisms through the application of the 16S rRNA gene sequencing methodology (25). In the Australia, *C. burnetii* was identified in 12.50% of tested blood samples and 1.70% of tested urine speciments through the use of quantitative real-time PCR (26). Since the contamination level in our study was lower compared to the studies conducted in the last one or two years, this difference can be attributed to the different husbandry and care of horses, the state of the immune system of horses and the detection technique applied for *C. burnetii* identification. The results of other studies revealed that the incidence of Q fever was more pronounced in mares than in stallions and that age was also one of the most important factors in the incidence of *C. burnetii* (7, 27). In our study, the positive case of *C. burnetii* was found in a seven-year-old mare that had foaled twice. No positve results were detected from any of the stallions. This could potentially suggest a strong affinity of *C. burnetii* for the uterine and mammary tissues, as the prevalence of this bacterium was notably higher in these two tissues (28, 29).

Since Q fever's classification as a notifiable disease varies and comprehensive clinical data are limited, ticks and other hosts, including ruminants, may act as infection reservoirs for horses. The identification of *C. burnetii* in horse riders further indicates that horses can act as a possible reservoir for human

transmission. As a result, individuals with frequent horse interactions, including veterinary professionals and enthusiasts, are at a heightened risk of contracting the infection. Therefore, timely diagnostic testing of horses, along with preventive measures and treatment, is essential.

CONCLUSION

The findings of the current investigation indicated that *C. burnetii* may be detectable in horses despite the lack of definitive clinical signs. Consequently, horses might be regarded as a potential benign reservoir for *C. burnetii*, and their conceivable contribution to the dissemination of Q fever warrants careful consideration. Although only one positive case was reported in this study, the disease could be transmitted to other horses and even to humans in direct or indirect contact with them, especially in poorly managed farms. Furthermore, the outcomes of this investigation revealed that the real-time PCR assay was a simple and reliable assay for detecting the causative agent of Q fever, and it can be facilitates detection of *C. burnetii* in numerous types of samples. Future studies should focus on investigating the incidence of *C. burnetii* among horse owners, and exploring strategies to improve on-farm management practices, such as enhanced biosecurity measures and regular testing, to reduce the risk of Q fever transmission. This can have a significant practical influence on reducing the disease burden and protecting human and animal health.

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REFERENCES

1. Eldin C, Mélenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. *Clin Microbiol Rev* 2017; 30: 115-190.
2. Gharban HA, Yousif AA. Serological and molecular phylogenetic detection of *Coxiella burnetii* in lactating cows, Iraq. *Iraqi J Vet Med* 2020; 44: 42-50.
3. Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, Van Rotterdam B, et al. Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiol Infect* 2009; 137: 1283-1294.
4. Nokhodian Z, Feizi A, Ataei B, Hoseini SG, Mostafavi E. Epidemiology of Q fever in Iran: A systematic review and meta-analysis for estimating serological and molecular prevalence. *J Res Med Sci* 2017; 22: 121.
5. Guatteo R, Seegers H, Taurel A-F, Joly A, Beaudeau F. Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. *Vet Microbiol* 2011; 149: 1-16.
6. Jaferi M, Mozaffari A, Jajarmi M, Imani M, Khalili M. Serologic and molecular survey of horses to *Coxiella burnetii* in East of Iran a highly endemic area. *Comp Immunol Microbiol Infect Dis* 2021; 76: 101647.
7. Khademi P, Ownagh A, Ataei B, Kazemnia A, Eydi J, Khalili M, et al. Molecular detection of *Coxiella burnetii* in horse sera in Iran. *Comp Immunol Microbiol Infect Dis* 2020; 72: 101521.
8. Marenzoni ML, Stefanetti V, Papa P, Casagrande Proietti P, Bietta A, Coletti M, et al. Is the horse a reservoir or an indicator of *Coxiella burnetii* infection? Systematic review and biomolecular investigation. V*et Microbiol* 2013; 167: 662-669.
9. Tehrani M, Ownagh A. Genomic detection of *Coxiella burnetii* based on plasmid genes in horses. *Vet Res Forum* 2023; 14: 317-322.
10. Metters G, Hemsley C, Norville I, Titball R. Identification of essential genes in *Coxiella burnetii*. *Microb Genom* 2023; 9: mgen000944.
11. Zhang Y, Fu J, Liu S, Wang L, Qiu J, van Schaik EJ, et al. *Coxiella burnetii* inhibits host immunity by a protein phosphatase adapted from glycolysis. *Proc Natl Acad Sci U S A* 2022; 119(1): e2110877119.
12. Berri M, Laroucau K, Rodolakis A. The detection of *Coxiella burnetii* from ovine genital swabs, milk and fecal samples by the use of a single touchdown polymerase chain reaction. *Vet Microbiol* 2000; 72: 285-293.
13. Vincent GA, Graves SR, Robson JM, Nguyen C, Hussain-Yusuf H, Islam A, et al. Isolation of *Coxiella burnetii* from serum of patients with acute Q fever. *J Microbiol Methods* 2015; 119: 74-78.
14. Schneeberger PM, Hermans MH, van Hannen EJ, Schellekens JJ, Leenders AC, Wever PC. Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. *Clin Vaccine Immunol* 2010; 17: 286-290.
15. Mohabbati Mobarez A, Bagheri Amiri F, Esmaeili S. Seroprevalence of Q fever among human and animal in Iran; A systematic review and meta-analysis. *PLoS*

Negl Trop Dis 2017; 11(4): e0005521.
16. Mohabati Mobarez A, Baseri N, Khalili M, Mostafavi E, Esmaeili S. Genotyping and phylogenetic analysis of *Coxiella burnetii* in domestic ruminant and clinical samples in Iran: insights into Q fever epidemiology. *Sci Rep* 2023; 13: 20374.
17. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, et al. Q fever in humans and farm animals in four European countries, 1982 to 2010. *Euro Surveill* 2013; 18: 20407.
18. Esmaeili S, Golzar F, Ayubi E, Naghili B, Mostafavi E. Acute Q fever in febrile patients in northwestern of Iran. *PLoS Negl Trop Dis* 2017; 11(4): e0005535.
19. Ghasemian R, Mostafavi E, Esmaeili S, Arabsheybani S, Davoodi L. Epidemiologic investigation of acute Q fever in North of Iran. *J Mazandaran Univ Med Sci* 2019; 29: 100-106.
20. Moradnejad P, Esmaeili S, Maleki M, Sadeghpour A, Kamali M, Rohani M, et al. Q fever endocarditis in Iran. *Sci Rep* 2019; 9: 15276.
21. Nourollahi Fard SR, Omid Ghashghaei O, Khalili M, Sharifi H. Tick diversity and detection of *Coxiella burnetii* in tick of small ruminants using nested Trans PCR in Southeast Iran. *Trop Biomed* 2016; 33: 506-511.
22. Esmaeili S, Mohabati Mobarez A, Khalili M, Mostafavi E, Moradnejad P. Molecular prevalence of *Coxiella burnetii* in milk in Iran: a systematic review and meta-analysis. *Trop Anim Health Prod* 2019; 51: 1345-1355.
23. Desjardins I, Joulié A, Pradier S, Lecollinet S, Beck C, Vial L, et al. Seroprevalence of horses to *Coxiella burnetii* in an Q fever endemic area. *Vet Microbiol* 2018; 215: 49-56.
24. Fenollar F, Fournier PE, Raoult D. Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infection. *J Clin Microbiol* 2004; 42: 4919-4924.
25. Seo M-G, Lee S-H, VanBik D, Ouh I-O, Yun S-H, Choi E, et al. Detection and genotyping of *Coxiella burnetii* and *Coxiella*-like bacteria in horses in South Korea. *PLoS One* 2016; 11(5): e0156710.
26. Tozer SJ, Lambert SB, Strong CL, Field HE, Sloots TP, Nissen MD. Potential animal and environmental sources of Q fever infection for humans in Queensland. *Zoonoses Public Health* 2014; 61: 105-112.
27. de Rooij MM, Schimmer B, Versteeg B, Schneeberger P, Berends BR, Heederik D, et al. Risk factors of *Coxiella burnetii* (Q fever) seropositivity in veterinary medicine students. *PLoS One* 2012; 7(2): e32108.
28. Kopecny L, Bosward KL, Shapiro A, Norris JM. Investigating *Coxiella burnetii* infection in a breeding cattery at the centre of a Q fever outbreak. *J Feline Med Surg* 2013; 15: 1037-1045.
29. Ndeereh D, Muchemi G, Thaiyah A, Otiende M, Angelone-Alasaad S, Jowers MJ. Molecular survey of *Coxiella burnetii* in wildlife and ticks at wildlife–livestock interfaces in Kenya. *Exp Appl Acarol* 2017; 72: 277-289.