The first serosurvey of equine coxiellosis in Iran

CURRENT STATUS: POSTED

Ali Asghar Mozaffari
Shahid Bahonar University of Kerman

Corresponding Author
aliasghar_mozaffari@uk.ac.ir
ORCiD: https://orcid.org/0000-0001-9468-6944

Mehdi Jaferi
Shahid Bahonar University of Kerman

Mohammad Khalili
Shahid Bahonar University of Kerman

Zahra Alinia
Shahid Bahonar University of Kerman

DOI: 10.21203/rs.2.12231/v1

SUBJECT AREAS
  Small Animal Medicine   Large Animal Medicine

KEYWORDS
  Coxiella burnetii, Horse, Iran, Q fever, Serology.
Abstract
Background: Q fever is a hyper endemic zoonosis in middle east countries. In Iran, serologic studies have been done in cattle, sheep, goat, camel and human. There is any report of Q fever in horse populations and this study is the first serosurvey in this area. Results: A total of 177 serum samples from North and South of Iran were randomly collected and were analyzed for Q fever by a commercial ELISA kit. Anti C.burnetii antibodies were detected in 5.64% of sera samples. Results of this study indicated that there was no significant difference in seroprevalence in different sexes, ages and breed groups. Conclusions: This is the first report of Q fever in horses of Iran and our study shows that the impact of Q fever disease on horses' health status in the studied area appears to be important. However, Additional studies are needed to assess whether the horse can be used as a relevant transmission risk indicator for Q fever in this area. Keywords: Coxiella burnetii, Horse, Iran, Q fever, Serology.

Background
Q fever is a zoonosis caused by Coxiella burnetii, a compulsory intracellular gram - negative bacterium capable of infecting both a wide range of animals and humans [1]. The organism is distributed worldwide, although serological surveys in New Zealand found no evidence of infection [2]. The seroprevalence of Q fever in regions and individual herds or flocks can be wide – ranging [3]. The horse's role as a C. burnetii reservoir infection was not adequately investigated. Serological evidence of C. burnetii infection in horses has long been reported. Study results were variable, ranging from non - detection of any evidence of infection [4] to An estimated 52.5 percent seroprevalence [5]. In addition, C. burnetii was suggested by a recent report as a potential abortifacient in French horses [6]. However, it remains unclear how horses are involved in the Q fever epidemiology [7, 8]. In fact, the few serological surveys available [5, 7, 9, 10] Suggest that horses can be exposed to C. burnetii naturally, but these studies are old and conducted with less sensitive serological tests than ELISA tests [11]. Furthermore, C. burnetii DNA Occasionally, was reported in samples of aborted equine or placental fetuses [6].

Serological studies in cattle, sheep, goats, camels and humans have been carried out in Iran [12-19].
Just as recently reported cases of human Q fever from Iran [19].

There is no report on serology of Q fever in horses in Iran based on our knowledge. We studied the presence of anti - *C. burnetii* antibodies in horses in certain locations in Iran and showed that the impact of Q fever disease on horses' health status in the studied area.

**Results**

Numbers of total serum and seropositive samples were presented in Table 1 in different locations, sex, breed and age groups. Anti - *C. Burnetii* antibodies were detected in 10 horses (8 females and 2 males), 9 horses belonging to the province of North Khorasan and 1 horse belonging to the province of Iran (p=0.026). The overall seroprevalence rate for horses was 5.64% (95% CI=2.7-10.1). Results of this study indicated that there was no significant difference in seroprevalence in different sexes, age and breed groups.

**Discussion**

Based on our knowledge, there is no report on Q fever serology in Iran's horses and this study is Iran's first research. The overall seroprevalence rate for horses in this study was 5.64 percent.

Seroprevalence of *C. burnetii* among horses in Poland and Brazil revealed that Polish and Brazil horses are seronegative [20, 21]. Desjardins and et al. assessed horse exposure in an area known to be endemic to ruminants and humans and reported 4% seropositivity in 2015 and 12% in 2016. [11]. Of the 816 samples of equine blood collected in South Korea between 2007 and 2013, 11 (1.3 %) were identified as *C. burnetii* by ELISA [22]. Marenzoni and et al. described a 15.8 percent seroprevalence in Italian horses [7]. A sero - epidemiological survey of California's domestic animals showed that 31 (26 %) of 121 horses had antibodies to *C. burnetii* [23]. Due to heterogeneity in terms of the specificity and sensitivity of the serological methods used (complementary fixation, seroagglutination and various ELISA tests), it remains difficult to compare our results with other serological surveys conducted on horses. In this study, Anti *C. burnetii* antibodies in 10 horses (8 females and 2 males) were detected, meaning females were more affected but the difference was not significant (p=0.097). Seo and et al. reported no significant difference in seropositivity between male and female horses [22]. Janati Pirouz et al. pointed out that female camels were more affected than males [24].
Anti *C. Burnetii* antibodies in current research were detected in 10 horses (8 females and 2 males), 9 horses belonging to the province of North Khorasan and 1 horse belonging to the Kerman province of Iran. \( p=0.026 \). Horses are kept with goats in North Khorasan and given the rate of abortion in these goats; infectious agents such as Q fever can be easily transmitted from goats to horses. Bamberg and et al described a Q-fever outbreak associated with a horse-boarding ranch that had purchased two herds of goats. This Q-fever outbreak was caused by exposure to infected goats. A cohort analysis between ranchers and persons revealed statistically significant associations between goat contact and seropositivity \[25\]. In goats, several studies also show that latently infected but seemingly healthy goats can also shed the organism in their vaginal secretions \[26\].

Results of this study indicated that there was no significant difference in seroprevalence rates in different age \( p=0.44 \) and breed groups \( p=0.06 \); however, seroprevalence rates were higher in Torkaman horses. In this topic, there is any report compared to our results.

It is necessary to consider horses as reservoirs for *C. Burnetii*. The detection of *C. burnetii* DNA-positive placentas in horses in the northern part of the Netherlands indicates that the contaminated environment has a true reservoir rather than a spillover effect in the south. Horses had previously been discussed as a risk factor in the outbreak of Q fever \[8\]. Detection of *C. burnetii* in equine aborted fetuses and neonates, suggested that *C. burnetii* together with other common pathogens, should probably be considered in equine abortion\[6\].

**Conclusion**

Serological results of present study provide indirect evidence that *C. burnetii* has been transmitted to horses in Iran. Our study shows that the impact of Q fever disease on horses' health status in the studied area appears to be low, but quite important. Additional studies are needed to assess whether the horse can be used as a relevant transmission risk indicator for Q fever in this area. Horses are typically a domestic species at the interface of infected domestic ruminants with humans, particularly in the areas studied.

One of the limitations of our study was the small number of positive cases that made it impossible for us to analyze statistics properly risk and epidemiological factors. The combination of molecular and
serological testing provides the best understanding of the significance of Q fever in the horses’ population.

**Methods**

Between 2017 and 2018, a total of 177 serum samples (77 from Kerman and 100 from North Khorasan provinces of Iran) were collected from 19 randomly selected horse herds (10 animals per herd) in South-East (province of Kerman) and North-East (province of North Khorasan). The animals were owned by individual farm and we obtained written informed consent to use the animals in our study from the owner(s) of the animals. After blood sampling animals were released in their farms. Because of inadequacy, 13 samples (including 13 herds) were removed. The sampled animals were males (57) and females (120), 1 - 16 years of age. Blood was collected through jugular vein puncture into sterile tubes. Blood samples were centrifuged and sera were gathered and stored at -20°C.

Antibodies against *C. Burnetii* in sera were detected using c-ELISA (ID Vet, France) as per the manufacturer's instructions. Descriptive statistics (crosstabs) (SPSS v18, SPSS Inc, Chicago, Illinois) have been used to compare data on seroprevalence rates among different age, sex, breed and geographic groups.

**Abbreviations**

*C. Burnetii*: *Coxiella burnetii*; ELISA: enzyme-linked immunosorbent assay; Q: query; SPSS: Statistical Package for the Social Sciences; Inc: incorporated

**Declarations**

**Ethics approval and consent to participate**: Institutional Research Ethics Committee, Veterinary Faculty of Shahid Bahonar University of Kerman, confirmed that the project was found to be in accordance to the ethical principles and the national norms and standards for conducting biological research in Iran. The approval ID is IR.UK.VETMED.REC.1398.013.

**Consent for publication**: Not applicable

**Availability of data and material**: The raw data collected during the present study are available from the corresponding author upon request.

**Competing interests**: Not applicable

**Funding**: The study was funded by the Research Council of Shahid Bahonar University of Kerman
The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors’ contributions: MJ and ZA gathered the samples, AAM and MK analyzed and interpret the samples, AAM wrote the article. All authors read and approved the manuscript.

Acknowledgements: This research was supported by Research Council of Shahid Bahonar University of Kerman and we thank them because of their support.

References

1. Rodolakis A: Q fever, state of art: Epidemiology, diagnosis and prophylaxis. *Small Ruminant Research* 2006, 62(1):121-124.

2. Constable PD, Hinchcliff KW, Done SH, Grünberg W: Veterinary medicine-e-book: a textbook of the diseases of cattle, horses, sheep, pigs and goats: Elsevier Health Sciences; 2017.

3. Guatteo R, Seegers H, Taurel A-F, Joly A, Beaudeau F: Prevalence of Coxiella burnetii infection in domestic ruminants: a critical review. *Veterinary microbiology* 2011, 149(1-2):1-16.

4. Raseta B, Mihajlovic B: Q-groznica kod domacih zivotinja u sap vojvodini. *Veterinarski glasnik* 1983.

5. Joshi M, Padbidri V, Rodrigues F, Gupta N: Prevalence of Coxiella burnetii infection among humans and domestic animals of Rajasthan State, India. *Journal of hygiene, epidemiology, microbiology, and immunology* 1979, 23(1):67-73.

6. Leon A, Richard E, Fortier C, Laugier C, Fortier G, Pronost S: Molecular detection of Coxiella burnetii and Neospora caninum in equine aborted foetuses and neonates. *Preventive veterinary medicine* 2012, 104(1-2):179-183.

7. Marenzoni ML, Stefanetti V, Papa P, Proietti PC, Bietta A, Coletti M, Passamonti F, Henning K: Is the horse a reservoir or an indicator of Coxiella burnetii infection? Systematic review and biomolecular investigation. *Veterinary*
microbiology 2013, 167(3-4):662-669.

8. Roest HI, van Solt CB, Tilburg JJ, Klaassen CH, Hovius EK, Roest FT, Vellema P, van den Brom R, van Zijderveld FG: Search for possible additional reservoirs for human Q fever, The Netherlands. Emerging infectious diseases 2013, 19(5):834.

9. Raseta B, Mihajlovic B: Q-fever in domestic animals in the province of Vojvodina. Veterinarski Glasnik 1983, 37(9):695-704.

10. Lang GH: Coxiellosis (Q fever) in animals. In: Q Fever: the disease. Volume 1, edn. Edited by Marrie Tj. Florida , USA: CRC Press; 1990: 23-48.

11. Desjardins I, Joulié A, Pradier S, Lecollinet S, Beck C, Vial L, Dufour P, Gasqui P, Legrand L, Edouard S: Seroprevalence of horses to Coxiella burnetii in an Q fever endemic area. Veterinary microbiology 2018, 215:49-56.

12. 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. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences, 22.

13. Sakhaee E, Khalili M: The first serologic study of Q fever in sheep in Iran. Tropical animal health and production 2010, 42(7):1561-1564.

14. Asadi J, Kafi M, Khalili M: Seroprevalence of Q fever in sheep and goat flocks with a history of abortion in Iran between 2011 and 2012. Vet Ital, 49(2):163-168.

15. Mobarez AM, Amiri FB, Esmaeili S: Seroprevalence of Q fever among human and animal in Iran; A systematic review and meta-analysis. PLoS neglected tropical diseases, 11(4):e0005521.

16. Ezatkhah M, Alimolaei M, Khalili M, Sharifi H: Seroepidemiological study of Q fever in small ruminants from Southeast Iran. Journal of infection and public
17. Esmaeili S, Mostafavi E, Shahdordizadeh M, Mahmoudi H: A seroepidemiological survey of Q fever among sheep in Mazandaran province, northern Iran. *Annals of Agricultural and Environmental Medicine*, 20(4).

18. Naderipour Z, Golchin M, Khalili M: Design of an ELISA kit for detection human acute Q fever. *Iranian Journal of Medical Microbiology*, 8(2):28-34.

19. Esmaeili S, Mobarez AM, Khalili M, Mostafavi E, Moradnejad P: Genetic evidence of *Coxiella burnetii* infection in acute febrile illnesses in Iran. *PLOS Neglected Tropical Diseases* 2019, 13(2):e0007181.

20. Szymańska-Czerwińska M, Jodełko A, Pluta M, Kowalik S, Niemczuk K: Seroprevalence of *Coxiella burnetii* among domestic ruminants and horses in Poland. *Acta virologica* 2017, 61(3):369-371.

21. Mares-Guia MAMd, Rozental T, Guterres A, Gomes R, Almeida DNd, Moreira NS, Barreira JD, Favacho AR, Santana AL, Lemos ERsd: Molecular identification of the agent of Q fever—*Coxiella burnetii*—in domestic animals in State of Rio de Janeiro, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical* 2014, 47(2):231-234.

22. Seo M-G, Lee S-H, VanBik D, Ouh I-O, Yun S-H, Choi E, Park Y-S, Lee S-E, Kim JW, Cho G-J: Detection and genotyping of *Coxiella burnetii* and *Coxiella*-like bacteria in horses in South Korea. *PLoS One* 2016, 11(5):e0156710.

23. Willeberg P, Ruppanner R, Behymer D, Haghhighi S, Kaneko J, Franti C: Environmental exposure to *Coxiella burnetii*: a sero-epidemiologic survey among domestic animals. *American Journal of Epidemiology* 1980, 111(4):437-443.

24. Janati Pirouz H, Mohammadi G, Mehrzad J, Azizzadeh M, Nazem Shirazi MH: Seroepidemiology of Q fever in one-humped camel population in northeast
Iran. Trop Anim Health Prod 2015 Oct;47(7):1293-8 doi: 101007/s11250-015-0862-z Epub 2015 Jun 13.

25. Bamberg WM, Pape WJ, Beebe JL, Nevin-Woods C, Ray W, Maguire H, Nucci J, Massung RF, Gershman K: Outbreak of Q fever associated with a horse-boarding ranch, Colorado, 2005. Vector-Borne and Zoonotic Diseases 2007, 7(3):394-402.

26. Agerholm JS: Coxiella burnetii associated reproductive disorders in domestic animals-a critical review. Acta Veterinaria Scandinavica 2013, 55(1):13.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

table 1.pdf