Published in final edited form as:
J Food Prot. 2017 April ; 80(4): 561–566. doi:10.4315/0362-028X.JFP-16-377.

Prevalence and Risk Factors of *Coxiella burnetii* Antibodies in Bulk Milk from Cattle, Sheep, and Goats in Jordan

MOHAMMAD M. OBAIDAT[1,*] and **GILBERT J. KERSH**[2]

[1]Department of Veterinary Pathology and Public Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Al Ramtha, Irbid, Jordan;

[2]Rickettsial Zoonoses Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30329, USA

Abstract

This large-scale cross-sectional study was conducted to determine the prevalence, geographical distribution, and risk factors for the presence of antibodies against *Coxiella burnetii* in bulk tank milk derived from dairy cattle, sheep, and goats in Jordan. Bulk milk samples were collected from 78 dairy cattle, 48 sheep, and 23 goat farms from various places in Jordan according to the density of these animal species in each region of the country. The samples were tested for *C. burnetii* antibodies using the CHEKIT Q-Fever Antibody ELISA kit. A standardized questionnaire was also used to collect data from each farm to identify and rank the risk factors for the presence of *C. burnetii* antibodies. The results revealed that 62.9% (95% confidence interval: 55.1 to70.0%) of the tested ruminant farms were positive for *C. burnetii* antibodies. Positive results were obtained from 70.9% (60.6 to79.5%) of dairy cattle farms, 52.1% (38.3 to 65.5%) of sheep farms, and 56.0% (37.1 to 73.3%) of goat farms. Six factors were associated with the presence of these antibodies on cattle farms, and five factors were associated with these antibodies on sheep and goat farms (chi-square test). The multivariate logistic regression model revealed that large dairy cattle farms, farms that add new animals to the herd, farms that infrequently clean the feeders, and farms in particular areas are 28.6, 19.9, 8.0, and 6.4 times more likely, respectively, to have animals with *C. burnetii* antibodies. Sheep and goat farms that mix their animals with those from other farms, graze more than 5 km, and infrequently sanitize the feeders were 8.0, 0.06, and 13.6 times more likely, respectively, to have animals with *C. burnetii* antibodies. These data reveal the widespread exposure of Jordanian ruminants to *C. burnetii* and suggest a high risk for public health.

Keywords

Farm animals; Middle East; Public health; Q fever

Q fever is a zoonotic disease that is caused by the gramnegative bacterium *Coxiella burnetii* and can result in significant morbidity and mortality in human populations (7, 28). Infection

*Author for correspondence. Tel: 962-7201000, Ext 22061; Fax: +962-2-7095117; mmobaidat@just.edu.jo.

with *C. burnetii* can also cause significant reproductive problems in some animal species. *C. burnetii* is transmitted to humans from animals via inhalation of aerosolized contaminated animal waste products. This pathogen can reach high levels in the placenta and birth products and is very stable in the environment because it forms a spore-like cell type known as the small cell variant, which resists harsh environmental conditions such as desiccation, osmotic stress, UV light, and disinfectants and is able to spread by wind among adjacent farms (10, 17, 29).

The animal reservoirs of *C. burnetii* are numerous and include many wild and domestic mammals, birds, and arthropods (15, 33). However, the primary reservoirs associated with human infection are cattle, sheep, and goats(23). Animals infected with *C. burnetii* usually are asymptomatic, but when clinical disease occurs, it is manifested as late abortion and reproductive disorders. However, animals can remain chronically infected and continue to shed the organism in milk and during subsequent pregnancies (1, 5, 24).

Despite the plethora of reports on the prevalence of *C. burnetii* in various countries worldwide, the majority of the published research was focused on the prevalence of serum antibodies in dairy animals rather than prevalence of antibodies in milk (14). Little information is available from small ruminants (sheep and goats) despite their significant role in human outbreaks (14). Thus, serological analysis of bulk tank milk can provide valuable and inexpensive information regarding herd exposure and prevalence of *C. burnetii* and is simpler to perform than serological tests on animal blood or serum (11, 12, 25, 31, 32, 39, 40, 45). A knowledge gap exists concerning the prevalence of *C. burnetii* in animals in Jordan and several Middle Eastern countries. This large-scale study was conducted to investigate the prevalence of antibodies against *C. burnetii* and associated risk factors for contamination with this pathogen in the milk of cattle, sheep, and goats in Jordan.

MATERIALS AND METHODS

Sample size.

The prevalence of *C .burnetii* infection in Jordan is unknown. At an assumed prevalence of 0.5 with an allowable error (95% confidence interval [CI]) around the estimate of 0.1, the required sample size would be 96 using the formula $n=z^2p(1 - p)/d^2$. In the present study, 159 farms were included to increase the power of the analysis; 86 cattle, 48 sheep, and 25 goat farms were randomly selected from various regions of Jordan for milk sample collection during 2015 and 2016.

Setting.

No vaccination program for Q fever currently exists for animals in Jordan. This cross-sectional study was designed to cover all 12 governorates of Jordan. Most (50 to 60%) of dairy cattle in Jordan are located in Al-Dulail, a small area in the northeast, whereas sheep and goat farms are spread throughout the country. Several farms randomly selected from each governorate in Jordan were included in the study. In each governorate, selected veterinarians helped with sample collection and administration of questionnaires because these professionals have strong ties of trust with their farmer clients. This approach was used to allow easier access for sample collection and to obtain accurate answers on the questionnaire.

Risk factors associated with *C. burnetii* infection.

A questionnaire was designed to collect data to assess the possible risk factors for *C. burnetii* infection. The questionnaire included questions about farm demographics, management, and disease incidence. The questionnaire was tested in a pilot study with a convenience sample of five farms, and all modifications were made before the questionnaire was finalized. The questionnaire was administered in Arabic by veterinarians took 5 min to complete. The repeatability of the questionnaire was examined by giving the same farmers the questionnaire in two forms: by face-to-face interview and by phone call. The repeatability was determined by calculating the agreement between answers to three questions: (i) How many animals are on your farm? (ii) Do you add or purchase new animals for your farm? (iii) How often do you clean your farm? The kappa scores for each question were 0.93 (very good),0.90 (very good), and 0.79 (good), respectively.

Milk sample collection and testing for *C. burnetii*.

A 100-mL sample of bulk or commingled milk was aseptically collected in sterile containers from each farm by trained veterinarians and transported daily under cold conditions in an ice box to the Food Hygiene Laboratories (Faculty of Veterinary Medicine, Jordan University of Science and Technology) for analysis.

Immediately upon arrival at the laboratory, the milk samples were centrifuged; the fat was discarded, and the nonfat milk serum was kept at −20°C until tested. The milk serum was tested for antibodies against *C. burnetii* using the Q-Fever Antibody Test Kit (product code 06-40669-04, IDEXX, Liebefeld-Bern, Switzerland) based on *C. burnetii* inactivated phase 1 and phase 2 antigens. This assay is considered superior, with higher sensitivity and specificity than complement fixation tests (2, 14, 25, 35, 39, 48). The optical densities (ODs) of all samples were determined in duplicate, averaged, and adjusted by subtracting the negative control OD.

The results were expressed as S/P values, which were estimated as the ratio between the sample (S) OD and the positive control (P) OD. The positive control was included in the test kit. The S/P values were estimated on a continuous scale with a theoretical range from zero to 8. According to the IDEXX instructions, an *S/P* value of <30% is considered negative, a value of ≥40% is considered positive, and values of 30 to 40% are considered intermediate.

Data management and statistical analyses.

The data were entered into an Excel (Microsoft, Redmond, WA) spreadsheet and analyzed using SPSS 2011, version 20 (SPSS Corp., IBM, Armonk, NY). A univariate analysis using a chi-square test was performed to determine the associations between a Q fever antibody–positive result (outcome variable) and potential risk factors. Potential risk factors with $P \leq 0.25$ (two tailed; alpha=0.25) and no collinearity ($r < 0.6$) and variance inflation factors of 5.0 were considered for further analysis. Collinearity between potential risk factors was examined using Spearman's rank correlations test. The overall prevalence was calculated as the total number of serologically positive samples divided by the total number of tested samples.

A multivariate model for *C. burnetii* antibody–positive status was constructed using a manual stepwise forward logistic regression analysis. Risk factors that were significant in the univariate model were used to construct the final multivariate logistic regression to study the potential risk collectively. A Hosmer-Lemeshow goodness-of-fit test was used to evaluate the fit of the models.

RESULTS

A total of 159 of ruminant farms were included in this study, and milk samples from 62.9% of the farms were positive for the presence of *C. burnetii* antibodies (95% CI,55.1 to 70.0%). The prevalence of these antibodies in bulk milk from cattle, sheep, and goats was 70.9% (60.6 to79.5%), 52.1% (38.3 to 65.5%), and 56.0% (37.7 to 73.3%), respectively.

The chi-square test for potential risk factors on cattle farms and on sheep and goat farms are shown in Tables 1 and 2, respectively. The risk factors for finding *C. burnetii* antibodies in cow's milk were farm location, herd size, history of abortion, adding new animals to the herd, practicing natural breeding, and frequency of farm cleaning. The risk factors for antibodies in ewe's and goat's milk were flock size, mixing animals with animals from other farms, grazing more than 5 km away from the farm, farm cleaning, and cleaning and sanitation of feeders.

The final model of the multivariate binary logistic regression for cattle is shown in Table 3. The model for cattle revealed that the probability of *C. burnetii* antibodies in bulk milk on large (>51 head) farms was 28.6 times higher than that on smaller farms. Farms that added new animals to the herd had a 19.9 times higher probability of antibodies in milk than did farms that did not add new animals. The probability of antibodies in farms on which the feeders were cleaned infrequently (monthly) was 8.0 times higher than that for farms on which feeders were cleaned frequently (weekly).

The final model of the multivariate binary logistic regression for sheep and goats is shown in Table 4. The probability of antibodies in the milk on sheep and goat farms that mix animals with animals from other farms was 8.0 times higher than that on farms that did not mix their animals with animals from other farms. Sheep and goats that grazed more than 5 km away from the farm were 0.06 times more likely to have *C. burnetii* antibodies than were animals that did not graze at this distance. The probability of finding*C. burnetii* antibodies in milk from sheep and goats from farms on which feeders were sanitized infrequently (less often than every 2 weeks) was 13.6 times higher than that from farms on which the feeders were sanitized frequently (every 2 weeks or more often).

DISCUSSION

Bulk milk samples were tested for *C. burnetii* antibodies in this study to obtain information about the prevalence of this pathogen on ruminant farms in Jordan. Taurel et al. (44) reported that the use of an enzyme-linked immunosorbent assay (ELISA) to analyze bulk tank milk could allow identification of herds with very low within-herd *C. burnetii* seropositivity. Not all seropositive animals shed *C. burnetii* in their milk, and shedding of this pathogen in milk is intermittent (13, 36, 37). Serological methods using animal blood and/or sera are useful for preliminary surveys of *C. burnetii* infection but do not allow identification of animals shedding *C. burnetii* because at least 25% of seronegative goats shed these bacteria (37). Some cattle and sheep shed *C. burnetii* before they develop antibodies, meaning that some infected animals do not seroconvert (15,48). Thus, testing bulk milk provides a better picture of the infection status on the whole farm and of the safety of dairy products made from unpasteurized milk, which is widely consumed in Middle Eastern countries such as Jordan (16).

In our study, 70.9% of the tested dairy cattle herds in Jordan were infected with *C. burnetii*, and antibodies were found in milk samples. A very high prevalence of *C. burnetii* (>90%) in bulk tank milk of dairy herds has been was reported in the United States (19). *C. burnetii* prevalence also has been reported in The Netherlands (79% (13) and 37.0% (24)), France (73.0% (13)), northern Spain (67% (6)), Denmark (59% (2)), northeastern Spain (49.4% (26)), southeastern Iran (54.4% (18)), and Ireland (37.9% (42)). The findings indicate that *C. burnetii* is widespread in cow's milk and perhaps may be explained by the longer period of *C. burnetii* shedding in cow's milk compared with ewe's and goat's milk (6, 36).

In this study, about 52.1 and 56.0% of the sheep and goat farms, respectively, were infected with *C. burnetii*, which indicates that *C. burnetii* is circulating in small ruminant farms in Jordan. In contrast, low *C. burnetii* prevalence was found in small ruminant bulk milk in The Netherlands. van den Brom et al. (45) tested bulk milk from sheep and goats using an ELISA and found prevalence values of 29.8 and 18.8%, respectively, with significant differences among different areas of The Netherlands. The prevalence of *C. burnetii* infection in small ruminants has also been determined in several countries based on results from animal serum samples. Therefore, comparison of our findings for antibodies in bulk milk with those of other studies must take this sample difference into consideration. The antibody prevalence in bulk milk is expected to be higher than the flock seroprevalence because not all animals are sampled in most flock seroprevalence studies (20, 38). The reported flock seroprevalence in goats differed among countries: 17.8, 42.9, 40.0, and 45% in The Netherlands, Spain, France, and Northern Ireland, respectively (13, 22,39). The flock seroprevalence for sheep was 89.0% in France and 14.5% in The Netherlands (13).

A higher prevalence of *C. burnetii* antibodies was found in bulk milk from dairy cattle than in bulk milk from sheep and goats, which was also reported in several countries. In central Portugal, the prevalence of *C. burnetii* antibodies in bulk milk from cattle (20%) was higher than that in milk from sheep and mixed herds (6%) (3). In contrast, several reports from the United States, Canada, Australia, and several European countries indicated that human outbreaks of Q fever associated with small ruminants were more frequent than those associated with cattle (11). Consequently, the prevalence was higher in sheep and goats than in cattle in some countries. For example, in Portugal the herd prevalence was higher in mixed sheep and goat herds(38.5%) and in sheep herds (37%) than in goat herds(28.8%) (4), and the herd seroprevalence in sheep (74%) was much higher than that in goats (45%) and cattle (43%) (39). In Bulgaria, goats have been the main reservoir for *C. burnetii* and have replaced cattle and sheep as the most frequent source of human infection (33). Higher seroprevalence in goats (48.2%) than in sheep (18.9%) and cattle (24%) was reported in Cyprus (33).

A significant difference in the prevalence of *C. burnetii* was found among different regions of Jordan for cattle only but not for sheep or goats in the present study. However, in other studies regional differences have been found within the same country. Georgiev et al. (13) reported that the prevalence difference was as high as fourfold among different areas of Bulgaria and even higher in some rural areas of Germany. Nusinovici et al. (27) found that the prevalence of *C. burnetii* in bulk milk of dairy cattle herds differed among regions of Sweden, where herds raised in regions with open landscape, high wind speed, high animal density, and high temperature had a higher risk of infection. The absence of differences among regions for sheep and goat farms reported in the present study might be attributed to the small area and by the similarity of the small ruminant husbandry systems in different regions of Jordan, but the close distances between farms in the Al-Dulail area might explain the higher prevalence in this area compared with the highlands.

In this study, an increase in herd for cattle, sheep, and goats was positively associated with *C. burnetii* infection. In several worldwide studies, the prevalence of *C. burnetii* in a given farm increased as the herd or flock size increased (2, 4, 22, 33, 41, 43, 46). The higher risk of introduction and/or transmission of *C. burnetii* in larger herds is possibly due to the higher number of calving or lambing females during the parturition season (47) and might be due to other management factors such as larger amounts of animal supplies such as feed and a higher number of people working at the farms (43). Therefore, larger herds are more prone to *C. burnetii* infection, and the number of animals must be considered a risk factor for *C. burnetii* dissemination.

In this study, infrequent cleaning and sanitation were significant risk factors for *C. burnetii* infection in dairy ruminants in Jordan. Similarity, van Engelen et al. (46) found that more frequent cleaning of barn bedding was inversely related to positive *C. burnetii* results in bulk tank milk. This inverse relationship might be attributed to a decreased transmission of *C. burnetii* to other animals in the same herd by eliminating contaminated birth material or vaginal excretions from the bedding.

Addition of new animals to the herd also was a significant risk factor for *C. burnetii* infection in the tested cattle farms in Jordan. Mixing animals with animals from other farms was a risk factor for infection in sheep and goat farms. These findings corroborate previous findings that purchase of cattle from at least two addresses was significantly and positively associated with anti–*C. burnetii* seropositivity in bulk tank milk of dairy cattle in The Netherlands (46).

The univariate analysis in this study revealed that the history of abortion was a significant factor for infection on sheep and goat farms but not on cattle farms. Other results have been reported regarding the association between reproductive problems or abortion with *C. burnetii* prevalence, i.e., positive associations between seroprevalence and abortion have been reported in sheep and cattle (8, 9). However, some researchers reported a lack of association between high seroprevalence of *C. burnetii* and reproductive disorders in cattle (6, 24, 34). In another study, infection with *C. burnetii* in cattle did not cause abortion. These findings suggest that infection can persist in cattle sometimes without producing significant clinical signs (30), but this might be not the case in sheep and goats.

The univariate analysis revealed that practicing natural breeding with bulls from the farm was a risk factor for *C. burnetii* infection. Little information is available about the probability of venereal transmission; however, in one study *C. burnetii* was reported in bull semen (21).

The high prevalence of *C. burnetii* detected in cattle, sheep, and goats in this study was attributed to several risk factors, and these findings highlight the significance of biosecurity and enhanced hygiene for decreasing the risk of *C. burnetii* contamination of bulk tank milk. Some of the risk factors, such as the increase in herd size for dairy cattle, are hard to control; thus, vaccination could be a suitable control measure for high-risk farms. In conclusion, this study confirms the widespread existence of *C. burnetii* antibodies in dairy cattle, sheep, and goats in Jordan and suggests that further studies on the public health consequences of *C. burnetii* shedding in ruminant milk would be beneficial.

ACKNOWLEDGMENTS

The authors thank Dr. Alaa Bani Salman for her support and technical advice and Dr. Saeed Hawamdeh, Eng. Anass Sawalgeh, and Hussam Jebreen for their help with the farm visits. This research was funded by the Deanship of Research at Jordan University of Science and Technology. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention or the U.S. Department of Health and Human Services.

REFERENCES

1. Agerholm JS 2013 Coxiella burnetii associated reproductive disorders in domestic animals—a critical review. Acta Vet Scand 55:13. doi:10.1186/1751-0147-55-13. [PubMed: 23419216]
2. Agger JF, Christoffersen AB, Rattenborg E, Nielsen J, and Agerholm JS. 2010 Prevalence of Coxiella burnetii antibodies in Danish dairy herds. Acta Vet Scand 52:5. doi: 10.1186/1751-0147-52-5. [PubMed: 20092653]
3. Anastacio S, Carolino N, Sidi-Boumedine K, and da Silva GJ. 2016 Q fever dairy herd status determination based on serological and molecular analysis of bulk tank milk. Transbound. Emerg. Dis 63:e293–e300. [PubMed: 25208655]
4. Anastacio S, Tavares N, Carolino N, Sidi-Boumedine K, and da Silva GJ. 2013 Serological evidence of exposure to Coxiella burnetii in sheep and goats in central Portugal. Vet. Microbiol 167:500–505. [PubMed: 24060100]
5. Arricau-Bouvery N, and Rodolakis A. 2005 Is Q fever an emerging or re-emerging zoonosis? Vet. Res 36:327–349. [PubMed: 15845229]
6. Astobiza I, Ruiz-Fons F, Pinero A, Barandika JF, Hurtado A, and Garcia-Perez AL. 2012 Estimation of Coxiella burnetii prevalence in dairy cattle in intensive systems by serological and molecular analyses of bulk-tank milk samples. J. Dairy Sci 95:1632–1638. [PubMed: 22459811]
7. Ben Amara A, Ghigo E, Le Priol Y, Lepolard C, Salcedo SP, Lemichez E, Bretelle F, Capo C, and Mege JL. 2010 Coxiella burnetii, the agent of Q fever, replicates within trophoblasts and induces a unique transcriptional response. PLoS One 5:e15315. [PubMed: 21179488]
8. Cabassi CS, Taddei S, Donofrio G, Ghidini F, Piancastelli C, Flammini CF, and Cavirani S. 2006 Association between Coxiella burnetii seropositivity and abortion in dairy cattle of northern Italy. New Microbiol 29:211–214. [PubMed: 17058789]

9. Cetinkaya B, Kalender H, Ertas HB, Muz A, Arslan N, Ongor H, and Gurcay M. 2000 Seroprevalence of coxiellosis in cattle, sheep and people in the east of Turkey. Vet. Rec 146:131–136. [PubMed: 10706331]

10. de Bruin A, Janse I, Koning M, de Heer L, van der Plaats RQ, van Leuken JP, and van Rotterdam BJ. 2013 Detection of Coxiella burnetii DNA in the environment during and after a large Q fever epidemic in the Netherlands. J. Appl. Microbiol 114:1395–1404. [PubMed: 23398323]

11. European Food Safety Authority, Panel on Animal Health and Welfare. 2010 Scientific opinion on Q fever. EFSA J 8:1595. doi:10.2903/j.efsa.2010.1595.

12. Gale P, Kelly L, Mearns R, Duggan J, and Snary EL. 2015 Q fever through consumption of unpasteurised milk and milk products—a risk profile and exposure assessment. J. Appl. Microbiol 118:1083–1095. [PubMed: 25692216]

13. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, Roest H, Stark K, Stegeman J, Vellema P, van der Hoek W, and More S. 2013 Q fever in humans and farm animals in four European countries, 1982 to 2010. Euro Surveill 18(8):pii=20407.

14. Guatteo R, Seegers H, Taurel AF, Joly A, and Beaudeau F. 2011 Prevalence of Coxiella burnetii infection in domestic ruminants: a critical review. Vet. Microbiol 149:1–16. [PubMed: 21115308]

15. Gyuranecz M, Denes B, Hornok S, Kovacs P, Horvath G, Jurkovich V, Varga T, Hajtos I, Szabo R, Magyar T, Vass N, Hofmann-Lehmann R, Erdelyi K, Bhide M, and Dan A. 2012 Prevalence of Coxiella burnetii in Hungary: screening of dairy cows, sheep, commercial milk samples, and ticks. Vector Borne Zoonotic Dis 12:650–653. [PubMed: 22651386]

16. Hilali M, El-Mayda E, and Rischkowsky B. 2011 Characteristics and utilization of sheep and goat milk in the Middle East. Small Ruminant Res 101:92–101.

17. Kersh GJ, Wolfe TM, Fitzpatrick KA, Candee AJ, Oliver LD, Patterson NE, Self JS, Priestley RA, Loftis AD, and Massung RF. 2010 Presence of Coxiella burnetii DNA in the environment of the United States, 2006 to 2008. Appl. Environ. Microbiol 76:4469–4475. [PubMed: 20472727]

18. Khalili M, Sakhaee E, Aflatoonian MR, and Shahabi-Nejad N. 2011 Herd-prevalence of Coxiella burnetii (Q fever) antibodies in dairy cattle farms based on bulk tank milk analysis. Asian Pac. J. Trop. Med 4:58–60. [PubMed: 21771417]

19. Kim SG, Kim EH, Lafferty CJ, and Dubovi E. 2005 Coxiella burnetii in bulk tank milk samples, United States. Emerg. Infect. Dis 11:619–621. [PubMed: 15829205]

20. Kittelberger R, Mars J, Wibberley G, Sting R, Henning K, Horner GW, Garnett KM, Hannah MJ, Jenner JA, Piggott CJ, and O'Keefe JS. 2009 Comparison of the Q-fever complement fixation test and two commercial enzyme-linked immunosorbent assays for the detection of serum antibodies against Coxiella burnetii (Q-fever) in ruminants: recommendations for use of serological tests on imported animals in New Zealand. N. Z. Vet. J 57:262–268. [PubMed: 19802039]

21. Kruszewska D, and Tylewska-Wierzbanowska S. 1997 Isolation of Coxiella burnetii from bull semen. Res. Vet. Sci 62:299–300. [PubMed: 9300554]

22. McCaughey C, Murray LJ, McKenna JP, Menzies FD, McCullough SJ, O'Neill HJ, Wyatt DE, Cardwell CR, and Coyle PV. 2010 Coxiella burnetii (Q fever) seroprevalence in cattle. Epidemiol. Infect 138:21–27. [PubMed: 19480726]

23. McQuiston JH, and Childs JE. 2002 Q fever in humans and animals in the United States. Vector Borne Zoonotic Dis 2:179–191. [PubMed: 12737547]

24. Muskens J, van Engelen E, van Maanen C, Bartels C, and Lam TJ. 2011 Prevalence of Coxiella burnetii infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. Vet. Rec 168:79. [PubMed: 21257587]

25. Niemczuk K, Szymanska-Czerwinska M, Smietanka K, and Bocian L. 2014 Comparison of diagnostic potential of serological, molecular and cell culture methods for detection of Q fever in ruminants. Vet. Microbiol 171:147–152. [PubMed: 24725446]

26. Nogareda C, Almeria S, Serrano B, Garcia-Ispierto I, and Lopez-Gatius F. 2012 Dynamics of Coxiella burnetii antibodies and seroconversion in a dairy cow herd with endemic infection and excreting high numbers of the bacterium in the bulk tank milk. Res. Vet. Sci 93:1211–1212. [PubMed: 22475008]

27. Nusinovici S, Frossling J, Widgren S, Beaudeau F, and Lindberg A. 2015 Q fever infection in dairy cattle herds: increased risk with high wind speed and low precipitation. Epidemiol. Infect 143:3316–3326. [PubMed: 25783480]
28. Omsland A, and Heinzen RA. 2011 Life on the outside: the rescue of Coxiella burnetii from its host cell. Annu. Rev. Microbiol 65:111–128. [PubMed: 21639786]
29. Oyston PC, and Davies C. 2011 Q fever: the neglected biothreat agent. J. Med. Microbiol 60:9–21. [PubMed: 21030501]
30. Paiba GA, Green LE, Lloyd G, Patel D, and Morgan KL. 1999 Prevalence of antibodies to Coxiella burnetii (Q fever) in bulk tank milk in England and Wales. Vet. Rec 144:519–522. [PubMed: 10378278]
31. Pearson T, Hornstra HM, Hilsabeck R, Gates LT, Olivas SM,Birdsell DM, Hall CM, German S, Cook JM, Seymour ML,Priestley RA, Kondas AV, Clark Friedman CL, Price EP, Schupp JM, Liu CM, Price LB, Massung RF, Kersh GJ, and Keim P. 2014 High prevalence and two dominant host-specific genotypes of Coxiella burnetii in U.S. milk. BMC Microbiol 14:41. [PubMed: 24533573]
32. Pinero A, Barandika JF, Hurtado A, and Garcia-Perez AL. 2014 Evaluation of Coxiella burnetii status in dairy cattle herds with bulk-tank milk positive by ELISA and PCR. Transbound. Emerg. Dis 61:163–168. [PubMed: 23009342]
33. Psaroulaki A, Hadjichristodoulou C, Loukaides F, Soteriades E, Konstantinidis A, Papastergiou P, Ioannidou MC, and Tselentis Y. 2006 Epidemiological study of Q fever in humans, ruminant animals, and ticks in Cyprus using a geographical information system. Eur. J. Clin. Microbiol. Infect. Dis 25:576–586. [PubMed: 16915398]
34. Raoult D, Marrie T, and Mege J. 2005 Natural history and pathophysiology of Q fever. Lancet Infect. Dis 5:219–226. [PubMed: 15792739]
35. Rodolakis A 2006 Q fever, state of art: epidemiology, diagnosis and prophylaxis. Small Ruminant Res 62:121–124.
36. Rodolakis A, Berri M, Hechard C, Caudron C, Souriau A, Bodier CC, Blanchard B, Camuset P, Devillechaise P, Natorp JC, Vadet JP, and Arricau-Bouvery N. 2007 Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. J. Dairy Sci 90:5352–5360. [PubMed: 18024725]
37. Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T,Touratier A, and Rodolakis A. 2009 Coxiella burnetii shedding routes and antibody response after outbreaks of Q fever–induced abortion in dairy goat herds. Appl. Environ. Microbiol 75:428–433. [PubMed: 19011054]
38. Rousset E, Durand B, Berri M, Dufour P, Prigent M, Russo P, Delcroix T, Touratier A, Rodolakis A, and Aubert M. 2007 Comparative diagnostic potential of three serological tests for abortive Q fever in goat herds. Vet. Microbiol 124:286–297. [PubMed: 17532581]
39. Ruiz-Fons F, Astobiza I, Barandika JF, Hurtado A, Atxaerandio R, Juste RA, and Garcia-Perez AL. 2010 Seroepidemiological study of Q fever in domestic ruminants in semi-extensive grazing systems. BMC Vet. Res 6:3. [PubMed: 20089188]
40. Ruiz-Fons F, Astobiza I, Barandika JF, Juste RA, Hurtado A, and Garcia-Perez AL. 2011 Measuring antibody levels in bulk-tank milk as an epidemiological tool to search for the status of Coxiella burnetii in dairy sheep. Epidemiol. Infect 139:1631–1636. [PubMed: 21251348]
41. Ruppanner R, Riemann HP, Farver TB, West G, Behymer DE, and Wijayasinghe C. 1978 Prevalence of Coxiella burnetii (Q fever) and Toxoplasma gondii among dairy goats in California. Am. J. Vet. Res 39:867–870. [PubMed: 727592]
42. Ryan ED, Kirby M, Collins DM, Sayers R, Mee JF, and Clegg T. 2011 Prevalence of Coxiella burnetii (Q fever) antibodies in bovine serum and bulk-milk samples. Epidemiol. Infect 139:1413–1417. [PubMed: 21073765]
43. Schimmer B, Luttikholt S, Hautvast JL, Graat EA, Vellema P, and Duynhoven YT. 2011 Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009–2010. BMC Vet. Res 7:81. [PubMed: 22208798]
44. Taurel AF, Guatteo R, Joly A, and Beaudeau F. 2012 Relationship between the level of antibodies in bulk tank milk and the within-herd seroprevalence of Coxiella burnetii in cows. Epidemiol. Infect 140:1710–1713. [PubMed: 22074814]

45. van den Brom R, van Engelen E, Luttikholt S, Moll L, van Maanen K, and Vellema P. 2012 Coxiella burnetii in bulk tank milk samples from dairy goat and dairy sheep farms in the Netherlands in 2008. Vet. Rec 170:310. [PubMed: 22351793]
46. van Engelen E, Schotten N, Schimmer B, Hautvast JL, van Schaik G, and van Duijnhoven YT. 2014 Prevalence and risk factors for Coxiella burnetii (Q fever) in Dutch dairy cattle herds based on bulk tank milk testing. Prev. Vet. Med 117:103–109. [PubMed: 25239684]
47. Woldehiwet Z 2004 Q fever (coxiellosis): epidemiology and pathogenesis. Res. Vet. Sci 77:93–100. [PubMed: 15196898]
48. World Organisation for Animal Health. 2010 OIE terrestrial manual, chap. 2.1.12, NB. Version adopted by the World Assembly of Delegates of the OIE, May 2010. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.12_Q-FEVER.pdf. Accessed 15 April 2016.

TABLE 1.

Prevalence and risk factors of C. burnetii antibodies in bulk milk of dairy cattle farms in Jordan, 2015 to 2016

Variable	No. of farms	No. of positive farms	*P* value (χ^2 test)
Location			0.00
Al-Dulail	50	45	
Highlands	36	16	
Herd size			0.00
Small (1–50)	37	15	
Large (>51)	49	46	
Having designated parturition area			0.15
Yes	10	9	
No	76	52	
Separate aborted animals[a]			0.11
Sometimes	18	13	
Never	44	39	
Abortions occurred on the farm			0.00
Yes	59	50	
No	27	11	
Adding animals to the herd			0.00
Yes	33	15	
No	53	46	
Natural breeding			0.01
Yes	38	32	
No	48	29	
How often the farmer cleans the feeders			0.00
Weekly	36	18	
Monthly	50	43	

[a]Not all farmers reported the occurrence of abortions on their farms.

TABLE 2.

Prevalence and risk factors of C. burnetii antibodies in bulk milk of sheep and goat farms in Jordan, 2015 to 2016

Variable	No. of farms	No. positive farms	*P* value (χ^2 test)
Species			0.75
Goats	25	14	
Sheep	48	25	
Area			0.90
North	34	19	
Middle	13	7	
South	26	13	
Flock size			0.01
Small (1–50)	20	6	
Large (>51)	53	33	
Mixing sheep and goats on one farm[a]			0.33
Yes	32	20	
No	29	13	
Mixing animals with animals from other farms			0.00
Yes	32	24	
No	41	15	
Using fences around the farm			0.98
Yes	45	24	
No	28	15	
Grazing more than 5 km away			0.03
Yes	14	11	
No	59	28	
Farm cleaning			0.04
Biweekly	9	2	
More than once per week	64	37	
Feeder cleaning and/or sanitation			0.00
Biweekly	30	7	
More than biweekly	43	32	
Water source			0.65
Well on the farm	31	18	
Spring or lake water	22	12	
Municipal water	20	9	
History of reproductive disorders			0.08
Yes	53	32	
No	19	7	

[a]Not all farmers raise both sheep and goats on the same farm.

TABLE 3.

Multivariate logistic regression model for variables associated with C. burnetii antibodies in bulk milk from dairy cattle farms in Jordan, 2015 to 2016

Variable	*P* value	Odds ratio	95% CI
Location (Al-Dulail)	0.05	6.35	1.4–35.5
Adding new animals to the herd (yes)	0.00	19.9	2.9–137.3
How often the farmer cleans the feeders (monthly)	0.02	8.0	1.5–42.4
Herd size (large)	0.00	28.6	3.5–230.9

TABLE 4.

Multivariate logistic regression model for variables associated with C. burnetii antibodies in bulk milk from sheep and goat farms in Jordan, 2015 to 2016

Variable	*P* value	Odds ratio	95% CI
Mixing animals with animals from another farm (yes)	0.004	8.0	2.1–30.8
Grazing more than 5 km away from the farm (yes)	0.00	0.06	0.01–0.40
Feeder sanitation (>biweekly)	0.00	13.6	3.4–54.6