Journal of Infection and Public Health 12 (2019) 190–194

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Seroprevalence and molecular detection of coxiellosis among cattle and their human contacts in an organized dairy farm

Pankaj Dhaka[a], Satyaveer S. Malik[a,*], Jay P. Yadav[a], Manesh Kumar[a], Amit Baranwal[b], Sukhadeo B. Barbuddhe[c], Deepak B. Rawool[a]

[a] Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute, Izatnagar, 243122, India
[b] Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, Izatnagar, 243122, India
[c] ICAR-National Research Centre on Meat, Chengicherla, Hyderabad, 500092, India

ARTICLE INFO

Article history:
Received 15 December 2017
Received in revised form
25 September 2018
Accepted 2 October 2018

Keywords:
Coxiella burnetii
Coxiellosis
Farm workers
Organized farm
Zoonosis

ABSTRACT

Background: The present investigation of *Coxiella burnetii* infection in cattle and farm workers on an organized cattle dairy farm, which appears to be the first of its kind in India, was undertaken to assess the status of this largely neglected and masked zoonosis.
Methods: A total of 665 samples comprising of serum (n = 224), milk (n = 217) and vaginal swabs (n = 224) collected from milch animals (n = 224) with a history of reproductive disorders were screened. Besides these, ticks (n = 114); animal feed (n = 4) and environmental samples (n = 13) as well as serum (n = 19) of farm workers were also collected. The animal sera and milk samples as well as human sera were tested for antibodies against *C. burnetii* by commercial ELISA kit, whereas, all the collected samples were subjected to trans-PCR targeting the *IS1111* gene of *C. burnetii*.
Results: A high positivity for coxiellosis was detected in sera (29.91%) and milk (26.73%) samples of dairy cattle as well as sera from human contacts (84.21%) by ELISA. The trans-PCR detected the pathogen in 12.94% sera, 14.73% vaginal swabs and 5.53% milk samples of cattle, and in one soil sample, however, the sera of the farm workers and tick were tested negative.
Conclusions: The high positivity for coxiellosis among cattle and farm workers highlight the need to undertake extensive epidemiological studies to unravel the trends of *C. burnetii* infection in India.

Introduction

Q fever is a highly infectious but largely 'neglected zoonosis' caused by Gram-negative bacterium, *Coxiella burnetii*, which infects a wide range of mammals including humans, birds and arthropods [1]. Globally ranked as one among the top 13 global priority zoonoses, it has been described as the most contagious disease [2] and is endemic in many parts of world [1], including India [3,4]. In developing countries, around 25% of the domestic ruminants exhibit signs of either current or past infection with *C. burnetii*, and are considered as major sources of infection to their human contacts [1,5]. The disease in ruminants is mainly asymptomatic, however, late abortions, stillbirths and other reproductive disorders can be observed in some cases [6]. On herd level at cattle farms, the estimated prevalence in bulk tank milk samples ranges from 10% to 94% [7,8]. It has been postulated that if a herd is infected with *C. burnetii*, the maintenance of infection is mainly attributed to circulation of the agent within the adult herd and that even asymptomatic bovine herds should be considered as the reservoirs of the bacteria capable of transmitting the disease to other animal species or even to humans [9,10].

Serological screening of humans and animals for the detection of *C. burnetii*-specific antibodies by ELISA has been found to be more sensitive and easy to perform, with results that are less subjective in nature [11]. However, use of PCR assay in combination with serology is indispensable for an early diagnosis of acute Q fever [12]. A PCR assay targeting insertion sequence (*IS 1111*) of *C. burnetii*, i.e., trans-PCR is considered as the test of choice for pathogen detection, as the transposon-like repetitive element of the pathogen with multiple genomic copies increase the sensitivity of test [13].

Coxiellosis may have an impact on public health in developing countries like India, especially in view of their peculiar socio-economic settings favoring frequent interactions between

* Corresponding author at: Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, 243122, India.
E-mail address: svsmalik@gmail.com (S.S. Malik).

https://doi.org/10.1016/j.jiph.2018.10.001

high-risk animals and their human contacts, most of which belong to the poor income groups. Moreover, the true picture of infection remains largely obscured due to limited diagnostic facilities and lack of public awareness about the disease [3,4,14]. It is noteworthy that in the past 60 years, there have only been about 25 publications on human and animal Q fever from India in global databases [4]. The objective of the study was to assess the status of coxiellosis in animals as well as their human contacts in an organized cattle dairy farm.

Material and methods

Study area and samples

A total of 665 samples comprising of serum (n = 224), milk (n = 217) and vaginal swabs (n = 224) were collected from cattle (n = 224) housed at an organized dairy farm, Bareilly, India during August, 2015 to February, 2016. Besides these, ticks (n = 114); animal feed (n = 4) and environmental samples (n = 13) as well as serum (n = 19) of farm workers serving for more than five years on the farm were also collected with informed consent. The selected farm animals (n = 224) were periodically screened for reproductive disorders (Supplementary Table 1) and different immunosuppressive infections (tuberculosis, Johne's disease and brucellosis). A high prevalence regarding these immunosuppressive infections was observed (unpublished data). Therefore, we assumed that the immunosuppressive diseases may pose a risk for *C. burnetii* infection to animals as it has been reported in humans [15]. A questionnaire covering important parameters pertaining to animal health and management, hygiene as well as risk factors for the human contacts was used for data collection (Table 1).

Clinical samples were collected in sterile containers. The samples of ticks (n = 114) from 16 infested cattle collected in sterile polypropylene sachets having 70% ethanol were identified morphologically as per the standard protocols. None of the animals on the farm was vaccinated for Q fever (Supplementary Table S1). The study has been performed in accordance with the Declaration of Helsinki and national ethical requirements.

Seroprevalence of animal and human coxiellosis by indirect ELISA

A commercial indirect ELISA Kit (Rochefort, Belgique) for the detection of antibodies against phase-I and phase-II antigens of *C. burnetii* in serum, plasma and milk samples of ruminants was used as per the manufacturer's instructions for screening the cattle serum and milk samples, while a commercial indirect ELISA kit (NovaTec Immundiagnostica GmbH, Germany) was employed for sero-screening of farm workers to detect IgG-class antibodies to phase-II antigen of *C. burnetii.*

Detection of C. burnetii *by trans-PCR assay*

Sample preparation for PCR

The DNA was extracted from clinical samples of cattle, i.e., sera, milk and vaginal swabs; associated ticks, serum samples of human contacts, as well as of feed and environmental samples of collected farm premises. The DNeasy Blood and Tissue Kit (Qiagen, USA) for DNA extraction was used for processing human and cattle sera as per the manufacturer's instructions, milk samples as described previously [16], tick samples as described elsewhere [17] and, environmental as well as feed samples as per described protocol [18]. The vaginal swabs were processed as per the method described [16] and the collected supernatant was used directly in PCR assay.

PCR protocol targeting IS1111 *gene*

The extracted DNA was subjected to the trans-PCR assay targeting the *IS1111* gene of *C. burnetii*, which was standardized as per the described protocol [3,16] with suitable modifications by employing Trans-1 (5′-TAT GTA TCC ACC GTA GCC AGT C-3′) and Trans-2 (5′-CCC AAC AAC ACC TCC TTA TTC-3′) oligonucleotides (Eurofins Genomics India Pvt. Limited, Bangalore) for pathogen detection based on the amplification of the 687 bp fragment. Briefly, 25 μL reaction mixture prepared for the trans-PCR assay included 2.5 μL of 10X PCR buffer (100 mM Tris–HCl buffer, pH 8.3 containing 500 mM KCl, 15 mM $MgCl_2$ and 0.01% gelatin), 200 μM of dNTP mix, 3.0 mM of $MgCl_2$, 2.0 μM of each primers, 0.5 Unit of Taq DNA polymerase (3 B Black Bio, Spain), 5 μL of DNA template and sterilized nuclease free water to make up the reaction volume. The DNA of *C. burnetii* Nine Mile phase 1 (strain RSA 493) was used as a positive control. The cycling conditions for PCR included an initial denaturation at 95 °C for 4 min followed by 35 cycles, each consisting of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 1 min. The final stage of the reaction included an extension of 10 min at 72 °C was performed in a Thermal cycler (Eppendorf, GmbH, Germany). The amplified PCR products along with 100 bp DNA ladder were resolved in 1% agarose gel containing ethidium bromide (10 $\mu g mL^{-1}$) by electrophoresis using Tris–Acetate-EDTA as running buffer and visualized by gel documentation system employing UVP Gel Seq software. Materials contaminated with ethidium bromide were disposed off according to the local guidelines.

Results

In the present study, the antibodies against *C. burnetii* could be detected by ELISA in 29.91% (67/224) of serum and 26.73% (58/217) of milk samples from cattle. Of these, 35.55% (32/90) had a history of reproductive disorders. The pathogen was detected in 12.94% (29/224) of cattle serum as well as 14.73% (38/224) of vaginal swabs and 5.53% (12/217) milk samples by trans-PCR (Supplementary Table S1). However, none of the ticks (n = 114) which belonged to *Boophilus* spp. and *Hyalomma* spp., tested positive for *C. burnetii*. On screening the feed (n = 4) and environmental samples (n = 13) by PCR, only one soil sample was found positive. The sero-screening of farm workers by ELISA revealed positivity for coxiellosis in 89.47% (17/19) subjects, however, none of the worker tested positive in PCR. The various possible observed risk factors among dairy animals and their human contacts are presented in Table 1.

Discussion

The prevalence of coxiellosis in recent times has increased many-fold, not only in human beings but also in animals [19]. Nevertheless, the information pertaining to the current status of this pathogen in ruminant species, including cattle is very scarce in India [3,4,14]. The present investigation appears to be the first systematically designed comprehensive approach for ascertaining the status of *C. burnetii* infection on an organized dairy farm in Indian context.

In India, the prevalence of coxiellosis in cattle remains grossly under diagnosed and under reported [3], and it has been reported to be in the range of 5.55% to 29.9% [3,14,20]. In present study, 29.91% seroprevalence of coxiellosis observed at animal level by ELISA commensurate with the median prevalence of *C. burnetii* infection among cattle globally reported as 19.4% at the animal level and 37.7% at the herd level [21].

The relatively high prevalence of coxiellosis in cattle observed as 29.91% in our study might be attributed to some of the observed risk factors (Table 1) as (i) large herd size (n = 224) with intensive farming practices, which has been reported to show a positive association of herd size with the *C. burnetii* infection in cattle [10,22]; (ii) hybrid herd (Frieswal) of exotic multiparous cattle on

Table 1
Analysis of important parameters pertaining to animal health and management, hygiene as well as risk factors for the human contacts.

Parameters	Response
Herds characteristics:	
• Type of herd (organized or unorganized)	Organized
• Breed (indigenous/exotic breed/hybrid)	Hybrid (Frieswal)
• Age and sex distribution	Multiparous female
• Mixed farming or typical herd	Typical herd (only cattle)
• Grazing system practiced (Extensive/Semi-extensive/Stall feeding)	Stall feeding
Housing Characteristics:	
• Quarantine procedure for purchased animals	Yes
• Biosecurity (presence of pets/vermins/birds/wild animals)	Not strict measures
• Type of housing (flooring- kucha/pucca)	Pucca (cemented)
• Ventilation (excellent/good/fair/poor)	Good
• Management of livestock waste/manure	Away from the shed but within premises
• Tick infestation on animals and/or presence of ticks in the environment	Frequent use of acaricides, scarcely presence of ticks (n = 114) only on 16 cattle
Individual animal characteristics:	
• Milk related traits-	
- Average milk production (herd average: liter/lactation)	3041.11
- Mastitis prevalence	8–10%
• Reproduction traits/history:	
- Abortions	31
- Repeat breeding	10
- Pre-mature still birth	29
- Retained placenta	39
- Dystocia	13
• Type and frequency of service	
- Artificial insémination	All
- Natural service	Nil
Calving practices:	
• By qualified vets/para-vets/local assistants/self	Both (veterinarian as well as para-vets)
• Use of calving box or in open	Calving box
• Isolation of the aborted cows	For 2–3 days
Disposal of placenta	
• Place of disposal	Away from the shed but within premises
• Method of disposal (use of disinfectant if any)	No use of disinfectant, directly discard on ground
• Time gap of disposal	Within hours of delivery
Disinfection practices followed:	
• Floor disinfection	Once in a month
• Hands disinfection	At the end of the day
• Farm equipments disinfection	Occasionally
Animal handlers behaviour:	
• Hygiene level	Poor to moderate
• Risky behaviors (unhealthy practices)	Drinking of raw milk, smoking, alcohol drinking and tobacco chewing

the farm under study, since older animals has been reported to have increased odds of getting infected, most often after the first calving [10]; (iii) improper disposal of biological wastes including placenta, aborted materials and dung on the farm, since aborted material of infected ruminant reservoirs has been opined to serve as the most important source of infection having up to 10^9 organisms per gram of placental tissue [23]; (iv) low biosecurity measures that allowed access of stray dogs and wild birds to the farm premises, especially when it has been reported that dog can be potentially infected by inhalation, tick bite, consumption of placenta or milk by infected ruminants and the wild mammals and birds can harbor the organism [24]; (v) high prevalence of reproductive disorders 40.18% (90/224) and other immunosuppressive infections like tuberculosis, brucellosis, Johne's disease as well as mastitis (8–10%) on the farm, which assume significance in the light of reported association of reproductive disorders [20,25] and subclinical mastitis [26] in dairy cattle with coxiellosis and immunosuppression in humans as important risk factor [15]; and (vi) infrequent floor and farm equipment disinfection as well as suboptimal personal and hand hygiene.

A high level of seropositivity (89.47%) in farm workers by ELISA but non-detection of pathogen in the sera by trans-PCR observed during the study might be indicative of chronic exposures to *C. burnetii* with immune clearance of pathogen [12]. The observed risk factors of coxiellosis for human contacts in our study included, (i) long contact with infected animals on the farm, since all the workers were engaged for more than five years, a time sufficient enough to expose them to the pathogen in such herd, as the *C. burnetii* infection once introduced, can remain within an animal population for at least 11 months [22]; (ii) large herd size (n = 224), since the potential risk factors for the human contacts have been considered as the dairy herds with more than 50 adult cows and 87.2% seroprevalence amongst the farmers in the Netherlands [27], over 80 animals in Danish farms and, above 200 animals in Latvian dairy farms [8,28]; (iii) ingestion of raw milk, as the contaminated raw milk or dairy products consumption has been reported to lead to seroconversion and perhaps, in a few cases, to Q fever [29]; (iv) low level of personal and hand hygiene practices followed by farm workers along with other unhealthy practices like, smoking, drinking and tobacco chewing, as these have been suggested to compromise the host defenses and to act as potential risk factors [30]. The findings of our study suggested that dairy farmers being one of the most exposed occupational groups to *C. burnetii* stand at high risk of acquiring *C. burnetii* infection, as they have regular contact with high-risk animals in dairy herds, especially the close contact during and after parturition [10].

In the diagnostic context, it is noteworthy that testing of only one type of biological sample may lead to misinterpretation with

regard to the shedding pattern of *C. burnetii*, and thereby, can underestimate the risk of transmission within the herd or contagion to their human contacts [31]. Therefore, we attempted to screen three biological samples of an individual cattle i.e., sera, milk and vaginal swabs for ascertaining the status of coxiellosis on a dairy farm in its right perspective.

In the present study, more number of animals revealed antibodies against *C. burnetii* in their serum (29.91%) and milk (26.73%) samples by ELISA as compared to the pathogen detection in their sera (12.94%), vaginal swabs (14.73%) and milk samples (5.53%) by the trans-PCR, which might be attributed to the enduring immunological response of these animals to *C. burnetii* leading to lower and/or intermittent shedding of the organism in the vaginal discharges and milk of cattle [8,32]. The low detection rate of pathogen in milk samples (5.53%) noticed in our study indicated the high variability and intermittent shedding of *C. burnetii* throughout the year [5]. It has been postulated that if a herd is infected with *C. burnetii*, then the maintenance of infection is mainly attributed to circulation of the agent within the adult herd and that even asymptomatic bovine herds should be considered as the reservoirs of the bacteria capable of transmitting the disease to other animal species or even to humans [9,10].

Ticks, though considered as an important vector in maintaining the bacterial survival in nature, are not the essential vectors for animal or human infection nor could these be regarded essential in the natural cycle of *C. burnetii* in livestock [33], and not all the ticks feeding on a *C. burnetii*-infected animal become infected [34]. In our study, negativity of all the tick samples (n = 114) screened for *C. burnetii* also indicated that the ticks might not be playing a significant role in the transmission of pathogen. Similar observations have been made by other researchers, who reported prevalence of *C. burnetii* in ticks to range from a very low [35,36] to nil [37]. The low recovery of ticks from animals (n = 16) might be attributed to the regular use of acaricides on the farm.

C. burnetii can exist for up to 150 days in soils [38], and is highly resistant to chemical disinfectants [39], therefore, the environment has also been suggested to serve as a significant source of contamination [40]. The detection of pathogen in a soil sample during the present study also reflected at the contamination of environment with the pathogen.

In conclusion, we reported for the first time high prevalence of antibodies against *C. burnetii* in dairy animals at an organized dairy farm. However, the positive serum samples were not subjected to other recommended serological test(s), therefore the possibility of some false positive result(s) cannot be ruled out. We also discussed the risk factors observed for *C. burnetii* infection in cattle and farm workers. Although the number of samples tested and the geographical range were fairly limited, it revealed a high probability of circulation of this most contagious but largely masked pathogen among animals and human contacts in organized dairy setting. More systematic studies at farm levels need to be conducted on a larger scale and in different geo-climatic zones for elucidating the ecology of *C. burnetii* infection, identification of the risk factors as well as implementation of appropriate biosecurity measures, which otherwise may adversely impact the livestock wealth and human health at dairy farm level.

Funding

No funding sources.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgements

The authors thank Director, Indian Veterinary Research Institute, Izatnagar for providing necessary facilities to undertake the research. We are grateful to Dr. Eric Ghigo, URMITE-IRD, Faculté de Médecine, France for providing the DNA of standard *C. burnetii* Nine Mile phase 1 (strain RSA 493). We thank Mr. K. K. Bhatt for his excellent technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jiph.2018.10.001.

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;30:115–90.
[2] Grace D, Mutua F, Ochungo P, Kruska R, Jones K, Brierley L, et al. Mapping of poverty and likely zoonoses hotspots. Zoonoses project 4. Report to the UK department for international development. Nairobi, Kenya: International Livestock Research Institute; 2012, p.119.
[3] Vaidya VM, Malik SVS, Bhilegaonkar KN, Rathore RS, Kaur S, Barbuddhe SB. Prevalence of Q fever in domestic animals with reproductive disorders. Comp Immunol Microb 2010;33:307–21.
[4] Kumar S, Gangoliya SR, Alam SI, Patil S, Ajantha GS, Kulkarni RD, et al. First genetic evidence of *Coxiella burnetii* in cases presenting with acute febrile illness. India J Med Microbiol 2017;66:388–90.
[5] Ruiz-Fons F, Astobiza I, Barandika JF, Hurtado A, Atxaerandio R, Juste RA, et al. Seroepidemiological study of Q fever in domestic ruminants in semi-extensive grazing systems. BMC Vet Res 2010;6:3.
[6] Arricau-Bouvery N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis? Vet Res 2005;36:327–49.
[7] Kim SG, Kim EH, Lafferty CJ, Dubovi E. *Coxiella burnetii* in bulk tank milk samples, United States. Emerg Infect Dis 2005;11:619.
[8] Boroduske A, Trofimova J, Kibilds J, Papule U, Sergejeva M, Rodze I, et al. *Coxiella burnetii* (Q fever) infection in dairy cattle and associated risk factors in Latvia. Epidemiol Infect 2017;145:2011–9.
[9] Parker NR, Barralet JH, Bell AM. Q fever. Lancet 2006;367:679–88.
[10] McCaughey C, Murray LJ, McKenna JP, Menzies FD, McCullough SJ, O'neill HJ, et al. *Coxiella burnetii* (Q fever) seroprevalence in cattle. Epidemiol Infect 2010;138:21–7.
[11] Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, Kersh GJ, et al. Diagnosis and management of Q fever — United States, 2013: recommendations from CDC and the Q fever working group. MMWR Recomm Rep 2013;62:1–30.
[12] 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;17:286–90.
[13] Denison AM, Thompson HA, Massung RF. *IS 1111* insertion sequences of *Coxiella burnetii*: characterization and use for repetitive element PCR-based differentiation of *Coxiella burnetii* isolates. BMC Microbiol 2007;7:91.
[14] Dhaka P, Malik SS, Yadav JP, Kumar M, Vergis J, Sahu R, et al. Seroscreening of lactating cattle for coxiellosis by trans-PCR and commercial ELISA in Kerala, India. J Exp Bio Agri Sci 2017;5:377–83.
[15] Lamas CDC, Rozental T, Bóia MN, Favacho ARM, Kirsten AH, Da Silva APM, et al. Seroprevalence of *Coxiella burnetii* antibodies in human immunodeficiency virus-positive patients in Jacarepaguá, Rio de Janeiro Brazil. Clin Microbiol Infect 2009;15:140–1.
[16] 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;72:285–93.
[17] Lalzar I, Harrus S, Mumcuoglu KY, Gottlieb Y. Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial communities. Appl Environ Microbiol 2012;78:4110–6.
[18] Fitzpatrick KA, Kersh GJ, Massung RF. Practical method for extraction of PCR-quality DNA from environmental soil samples. Appl Environ Microbiol 2010;76:4571–3.
[19] Gwida M, El-Ashker M, Khan I. Q fever: a re-emerging disease. J Vet Sci Technol 2012;3:2.
[20] Das DP, Malik SVS, Rawool DB, Das S, Shoukat S, Gandham RK, et al. Isolation of *Coxiella burnetii* from bovines with history of reproductive disorders in India and phylogenetic inference based on the partial sequencing of IS1111 element. Infect Genet Evol 2014;22:67–71.

[21] Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F. Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. Vet Microbiol 2011;149:1–16.
[22] Paul S, Agger JF, Markussen B, Christoffersen AB, Agerholm JS. Factors associated with *Coxiella burnetii* antibody positivity in Danish dairy cows. Prev Vet Med 2012;107:57–64.
[23] Bouvery NA, Souriau A, Lechopier P, Rodolakis A. Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes. Vet Res 2003;34:423–33.
[24] Porter SR, Czaplicki G, Mainil J, Guattéo R, Saegerman C. Q Fever: current state of knowledge and perspectives of research of a neglected zoonosis. Int J Microbiol 2016;2011:e–248418.
[25] Ortega-Mora LM. Is Q fever a significant cause of reproductive failure in cattle? Vet Rec 2012;170:257–8.
[26] Barlow J, Rauch B, Welcome F, Kim SG, Dubovi E, Schukken Y. Association between *Coxiella burnetii* shedding in milk and subclinical mastitis in dairy cattle. Vet Res 2008;39:23–32.
[27] Schimmer B, Schotten N, Engelen EV, Hautvast JLA, Schneeberger PM, van Duijnhoven YT. *Coxiella burnetii* seroprevalence and risk for humans on dairy cattle farms, the Netherlands, 2010-2011. Emerg Infect Dis 2014;3:417–25.
[28] Agger JF, Paul S. Increasing prevalence of *Coxiella burnetii* seropositive Danish dairy cattle herds. Acta Vet Scand 2014;56:46.
[29] Fishbein DB, Raoult D. A cluster of *Coxiella burnetii* infections associated with exposure to vaccinated goats and their unpasteurized dairy products. Am J Trop Med Hyg 1992;47:35–40.
[30] Daniele RP, Dauber JH, Altose MD, Dauber JH, Rowlands DT, Gorenberg DJ. Lymphocyte studies in asymptomatic cigarette smokers: a comparison between lung and peripheral blood. Am Rev Respir Dis 1977;116:997–1005.
[31] Guatteo R, Beaudeau F, Berri M, Rodolakis A, Joly A, Seegers H. Shedding routes of *Coxiella burnetii* in dairy cows: implications for detection and control. Vet Res 2006;37:827–33.
[32] Guatteo R, Beaudeau F, Joly A, Seegers H. *Coxiella burnetii* shedding by dairy cows. Vet Res 2007;38:849–60.
[33] Angelakis E, Raoult D. Q fever. Vet Microbiol 2010;140:297–309.
[34] Smith DJW, Derrick EH. Studies in the epidemiology of Q Fever. The Isolation of Six Strains of Rickettsia burnetii from the Tick Haemaphysalis humerosa. Aust J Exp Biol Med Sci 1940;18(pt. 1).
[35] Řeháček J, Urvölgyi J, Kocianova E, Sekeyová Z, Vavrekova M, Kováčová E. Extensive examination of different tick species for infestation with *Coxiella burnetii* in Slovakia. Eur J Epidemiol 1991;7:299–303.
[36] Toledo A, Jado I, Olmeda AS, Casado-Nistal MA, Gil H, Escudero R, et al. Detection of *Coxiella burnetii* in ticks collected from Central Spain. Vector-Borne Zoonotic Dis 2009;9:465–8.
[37] Astobiza I, Barral M, Ruiz-Fons F, Barandika JF, Gerrikagoitia X, Hurtado A, et al. Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. Vet Microbiol 2011;147:190–4.
[38] Welsh HH, Lennette EH, Abinanti FR, Winn JF. Air-borne transmission of Q fever: the role of parturition in the generation of infective aerosols. Ann Ny Acad Sci 1958;70:528–40.
[39] Scott GH, Williams JC. Susceptibility of *Coxiella burnetii* to chemical disinfectants. Ann Ny Acad Sci 1990;590:291–6.
[40] Nusinovici S, Hoch T, Brahim ML, Joly A, Beaudeau F. The effect of wind on *Coxiella burnetii* transmission between cattle herds: A mechanistic approach. Transbound Emerg Dis 2015;64:585–92.