Epidemiological study of *Coxiella burnetii* in dairy cattle and small ruminants in Québec, Canada.

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ABSTRACT

The bacterium *Coxiella burnetii* (*C. burnetii*) can infect a wide range of animals, most notably ruminants where it causes mainly asymptomatic infections and, when clinical, it is associated with reproductive disorders such as abortion. It is also the etiological agent of Q fever in humans, a zoonosis of increasingly important public health concern. A cross-sectional study was performed to estimate the apparent prevalence and spatial distribution of *C. burnetii* positivity in dairy cattle and small ruminant herds of two regions of Québec, Canada, and identify potential risk factors associated with positivity at animal and herd levels. In dairy cattle herds, individual fecal samples and repeated bulk tank milk samples (BTM) and were collected. In small ruminant herds, serum and feces were sampled in individual animals. ELISA analyses were performed on serum and BTM samples. Real-time quantitative PCR (qPCR) was done on fecal and BTM samples. An animal was considered *C. burnetii*-positive when at least one sample was revealed positive by ELISA and/or qPCR, while a herd was considered *C. burnetii*-positive when at least one animal inside that herd was revealed positive. None of the 155 cows had a qPCR-positive fecal sample, whereas 37.2 % (95 % CI = 25.3 - 49.1) of the 341 sheep and 49.2 % (95 % CI = 25.6 - 72.7) of the 75 goats were *C. burnetii*-positive. The apparent prevalence of *C. burnetii*-positive herds was 47.3 % (95 % CI = 35.6 - 59.3) in dairy cattle herds (n = 74), 69.6 % (95 % CI = 47.1 – 86.8) in sheep flocks (n = 23) and 66.7 % (95 % CI = 22.3 - 95.7) in goat herds (n = 6). No spatial cluster of positive herds was detected. At the individual level, the only significant association with positivity in multivariable regressions was higher parity number in small ruminants. At the herd level, the use of calving group pen, the distance to the closest positive bovine herd, and small ruminant herd density in a 5 km radius were associated with dairy cattle herd positivity, whereas small ruminant herds with more than 100 animals and with a dog on the farm had greater odds of *C. burnetii* positivity. Our study shows that the infection is frequent on dairy cattle and small ruminant herds from the two studied regions and that some farm and animal characteristics might influence the transmission dynamics of the *C. burnetii* infection.

1. Introduction

Coxiella burnetii is a Gram-negative obligate intracellular bacterium. It is the etiological agent of Q fever (query fever), a zoonotic disease in humans (Eldin et al., 2017). Its primary reservoirs are cattle, sheep, and goats. This pathogen is distributed worldwide and is mostly transmitted by inhalation of infected aerosols from animal sources (Eldin et al., 2017), most notably from the very high bacterial load in infected placentas and parturition fluids (Roest et al., 2012). Originally described as an occupational zoonosis, the large outbreak of 2007-2010 in the Netherlands with over 4000 notified human cases highlighted that Q fever was not restricted to slaughterhouses' workers, veterinarians and farmers but could be transmitted to the community and could pose major healthcare and public health problems (Schneeberger et al., 2014).

Q fever in humans is often asymptomatic but it can lead to a severe acute disease characterized by fever, headache and pneumonia. In pregnant women, the infection can cause various obstetrical complications including miscarriage. Persistent or chronic infections are also reported mostly in patients with valvular diseases and in immunocompromised people (Eldin et al., 2017). Similarly, in domestic ruminants, *C. burnetii* infection is usually asymptomatic. However, clinical cases of abortions are frequently documented and the infection is suspected to be associated with other reproductive disorders such as infertility, retained placenta and endometritis (Agerholm, 2013). Infected ruminants, especially sheep and goats, are known as heavy shedders of *C. burnetii*, particularly around abortion or parturition (Welsh et al., 1951; Roest et al., 2012). Several epidemiological studies supported by outbreak investigations have pointed out sheep, goats and dairy cattle as the main sources of human infections (Clark and Soares Magalhaes, 2018; Park et al., 2018; Woldeyohannes et al., 2018).

The transmission cycle of *C. burnetii* is complex and still not fully understood (Eldin et al., 2017). Once infected, ruminants can contribute to the dissemination of the bacteria within herds during parturition or abortion, via contaminated fetuses, fetal membranes or fluids, or by shedding the bacterium in feces, vaginal mucus or milk (Guatteo et al., 2007b; Rodolakis et al., 2007; Eldin et al., 2017). Many other species, including free-living amoebae, birds, wild and domestic mammals can become infected by the bacteria and could be a source of infection for domestic ruminants and humans (Maurin and Raoult, 1999; Eldin et al., 2017). Many risk factors have been associated with *C. burnetii* infection in ruminant herds, including herd size, type of production, biosecurity practices and presence of domestic carnivores (Schimmer et al., 2011; Paul et al., 2012; Agger et al., 2013; Paul et al., 2014). In addition, recent studies have shown that the local farm environment, such as a high regional herd density, open landscape, low soil moisture and high-speed wind conditions, can increase the risk of *C. burnetii* herd infection (Schimmer et al., 2011; Nusinovici et al., 2015).

In Canada, data on *C. burnetii* infection in the domestic ruminant populations are scarce (Lang, 1988; Lang et al., 1991; Hatchette et al., 2003; Meadows et al., 2015). The specific objectives of this study were *i)* to estimate the apparent prevalence of *C. burnetii*-positivity in dairy cattle and small ruminant herds based on the detection of antibodies by ELISA and/or bacterial DNA by quantitative PCR, *ii)* to determine whether spatial clusters of *C. burnetii*-positive herds were present, and *iii)* to identify potential risk factors associated with animal and herd positivity.

2. Material and methods

2.1. Study design and source population

A cross-sectional study was conducted on dairy cattle and small ruminant (sheep or goat) herds from May to October 2011 in two regions, Montérégie and Bas-St-Laurent, in Québec, Canada. Only herds with at least 15 adult animals were included.

2.2. Farm selection

The list of all registered farms located within the two regions was obtained from the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ). Dairy cattle herds with at least 15 breeding cows were selected at simple random with a target of 100 herds. This sample size was determined based on an expected *C. burnetii* herd-level positivity of 50 % with 95 % confidence and 10 % precision (Dohoo et al., 2009). Due to a limited number of small ruminant farms in the two regions, all small ruminant farms with a herd of at least 15 breeding animals were selected. Managers of selected farms were contacted by phone to solicit their participation in this study.

2.3. Animal selection and sample collection

2.3.1 Dairy cattle

On each participating farm, the herd veterinarian collected three bulk tank milk (BTM) samples, 3 to 5 weeks apart. The agitator inside the tank was activated for 5 to 10 minutes before sampling. Milk was collected using a sterile pipette and placed into a milk tube.

Among participating dairy cattle herds, a random subsample of 31 herds were selected for feces collection at the time of first BTM sampling. Five cows per herd were selected by herd veterinarians among females born on the farm and aged ≥ 6 months. Veterinarians were asked to calculate a selection step by dividing the lactating herd size by five, and to determine a way to order cows in the farm, which was in general the stall order. Then, they had to systematically select the cows according to this step, starting with the third cow (which was randomly determined). This sample size of 155 cows was calculated to detect at least one *C. burnetii*-positive animal in the sampled population with a 95 % confidence, given an expected apparent prevalence of 2 % and an estimated average herd size of 70 cows (Dohoo et al., 2009). Feces were collected directly from the rectum using clean disposable gloves and transferred into a sterile specimen container. The breed, age, number of days in milk and previous outdoor access of sampled cows were noted according to farm registers and/or information provided by the farm manager.

BTM and fecal samples were kept on ice and sent to the laboratory within 24 hours where they were stored at 4^{o} C until analysis.

2.3.2 Small ruminants

In each participating small ruminant herd, 15 breeding females aged ≥ 6 months and born on the farm were selected by herd veterinarians. A proportional stratified random sampling of animals by age group and reproductive stages (gestation, lactation or dry) was used, and the selected animals had to be distributed in at least three different pens. The sample size was calculated to detect at least one *C. burnetii*-positive animal per herd with a 95 % confidence, given an expected apparent prevalence of 20 % and an estimated herd size of 150 animals (Dohoo et al., 2009). The veterinarian collected 10 mL-blood samples by jugular venipuncture. Fecal samples were collected as described above for cows. The breed, parity, number of days in milk and previous outdoor access of each sampled animal was noted according to farm registers or herd manager. Blood and fecal samples were kept on ice and sent to the laboratory within 24 hours and stored as described above.

2.4. Questionnaire

Two questionnaires on herd characteristics and management practices were developed by the research team, one for dairy cattle and the other for small ruminants. The two questionnaires (in French) are available from the authors upon request. Questionnaires were reviewed by four veterinarians before their administration to ensure their clarity. Each questionnaire was administered by the herd veterinarian to the farm manager at time of sampling.

2.5. Regional animal density and farm proximity

The geographical coordinates of the main premise housing animals for each ruminant farm located in the two regions, along with the dairy cattle, ovine, and caprine inventory of each farm, were obtained from the MAPAQ and the spatial distribution of farms was mapped. For each herd included in our study, the distance to the closest *C. burnetii*-positive *i)* dairy cattle, *ii)* small ruminant, and *iii)* ruminant (dairy cattle or small ruminant) farm among the other farms sampled was calculated; the *C. burnetii* herd positivity was based on our case definition (section 2.7.1). The animal density per km^2 in a 1 km and 5 km radius of each farm included in our study was calculated based on farm inventories for cattle (dairy and beef), small ruminants, and all cattle and small ruminant farms combined (although our study focuses on dairy cattle and small ruminants, all type of bovine production were considered for animal density calculation as they can all be infected by *C. burnetii)*. All spatial analyses were performed in ArcGIS version 10.5 (Esri, Redlands, CA, USA).

2.6. Laboratory analyses

2.6.1. ELISA

BTM and serum samples were tested using the ID Screen® Q Fever Indirect Multi-species ELISA kit (ID.Vet, Grabels, France), coated with *C. burnetii* antigen from a bovine isolate which detects antibodies of phases I and II. BTM samples to positive control optical density ratio (S/P ratio) values were interpreted according to the manufacturer's instructions: negative (≤ 30 %), doubtful (> 30 to ≤ 40 %), or positive (> 40 %). For sera, S/P ratio values were interpreted as negative (≤40 %), doubtful (> 40 to ≤ 50 %) or positive (> 50 %). Doubtful ELISA results for BTM and serum samples were reclassified as positive for statistical analyses. The sensitivity and specificity of this ELISA kit in the context of a prevalence study were not available. The ELISA results were determined using the ELx808™ absorbance microplate reader (BioTek, Winooski, VT, United States).

2.6.2. Real-time quantitative PCR assay

Real-time quantitative PCR (qPCR) was performed to detect and quantify the presence of *C. burnetii* in samples. For BTM samples, 1 mL was centrifuged at full speed (13 600 rpm/16 800 g) for 30 minutes. The supernatant was discarded, and the pellet resuspended in 1 mL of PBS buffer. For feces samples, 1 g was resuspended in 5 mL of PBS buffer and vortexed for 60 seconds. A 200 µL volume of each suspension were subjected to DNA extraction using QIAamp DNA mini kit (Qiagen, Toronto, ON, Canada) following the manufacturer's recommendations and eluted in 50 µL of AE buffer. Five µL was used as template in the qPCR assay as previously described using primers and probe for the amplification and detection of the *icd* (Isocitrate dehydrogenase [NADP]) gene; (Klee et al., 2006). Positive (*C. burnetii* genomic DNA) and negative (H_2O) controls were included in each run. Samples showing *Cq* (cycle threshold) values

< 40 were considered positive. Using a calibration curve made with known quantity of the *icd* gene copies, the *Cq* values of positive samples were used to extrapolate the input *C. burnetii* genomic copy number within the tested samples. As previously shown for a similar PCR test, this assay has high analytic sensitivity and specificity (Klee et al., 2006).

2.7. Statistical analyses

2.7.1. Case definition

BTM were considered *C. burnetii*-positive when at least once classified positive by ELISA and/or qPCR. A *C. burnetii*-positive animal status was given to small ruminants with a positive ELISA (serum) or qPCR (BTM), or cows with a positive qPCR (feces). At the herd level, a *C. burnetii*-positive herd was defined as a herd in which at least one sample (BTM, serum, feces), among all samples collected on the herd, was positive to ELISA and/or qPCR.

2.7.2. Apparent prevalence estimation

Apparent prevalence of *C. burnetii*-positivity and the corresponding 95 % confidence intervals (CI) were estimated at the BTM sample, animal and herd levels for each ruminant category and type of sample (serum, feces) collected. Apparent prevalence estimates at the BTM sample level were adjusted for herd clustering, whereas they were adjusted for herd clustering and sampling weights at the animal level. When no positive was detected, the CIs were estimated using an exact estimation method. No adjustment was applied for sensitivity and specificity of the diagnostic tests given the absence of available information for the specific test used in the context of a prevalence study. The mean titer (in a log 10 scale) of *C. burnetii* among qPCR-positive dairy cattle BTM samples was estimated. . Analyses were conducted in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

2.7.3. Spatial cluster detection

Spatial clusters of *C. burnetii* positive farms were assessed using the Kulldorff circular spatial scan test based on a Bernoulli distribution, performed in SaTScan (Boston, MA, USA; Kulldorff, 1997). Analyses were done separately for dairy cattle and small ruminants, and for all species combined. Statistical significance of clusters was determined using 9999 Monte Carlo permutations.

2.7.4. Risk factors analysis – animal level

Multi-level logistic regression was used to model the risk of *C. burnetii* positivity according to our case definition in small ruminants, with goats and sheep combined. Maximum likelihood estimation based on Laplace approximation with herds included as a random effect was used (GLIMMIX procedure of SAS version 9.4; SAS Institute Inc.). Only animals from *C. burnetii*-positive herds were included in the analyses. Potential risk factors (parity, days in milk, previous outdoor access) were categorized. From the full multivariable model, a backward manual elimination of variables was performed with a *P*-value > 0.05 (likelihood ratio test) as criteria for rejection. However, these variables were kept in the model as potential confounders if their removal changed the coefficient value of another variable in the model by > 30 % (Dohoo et al., 2009). Odds ratios (OR) were used to present the results of the final model. As a sensitivity analysis to determine the potential impacts of the inclusion of doubtful results on final results, an alternative model was built by re-estimating the final model after exclusion of the animals with an

ELISA-doubtful and qPCR-negative status, as well as animals from herds with an ELISA-doubtful and qPCR-negative status.

2.7.5. Risk factors analysis – herd level

Logistic regressions were used to model the herd positivity to *C. burnetii* according to potential risk factors derived from the questionnaire and spatial analyses. For animal density and farm proximity continuous variables, linearity of the log odd assumption was visually assessed by categorizing the variable in quartiles, fitting a univariable logistic regression and plotting the predicted value against the average value of each category. Those variables not meeting the linearity assumption and all other potential risk factors were categorized based on information available in the literature or using medians for continuous variables, while ensuring that each level of variables included at least 10 % of the data. A first screening of the variables was performed using univariable logistic regressions; those with a *P*-value < 0.20 (likelihood ratio test) were kept for multivariable modeling. The correlation between these selected variables was assessed using chi-square tests. In the presence of strong correlation among variables, or when there was evidence of multicollinearity in further multivariate modeling, only the most relevant variable in a biological perspective was kept for multivariate modeling if one could be identified; otherwise, the variable with lowest *P*-value was retained. From the full multivariable model, a backward manual elimination of variables was used as described in section 2.7.3. For small ruminants, exact logistic regressions were used due to data scarcity. Fit of the final model (when based on maximum likelihood estimation) was assessed with the Hosmer-Lemeshow goodness-of-fit test. Odds ratios were used to present the results. As a sensitivity analysis to determine the potential impacts of the inclusion of doubtful results on final results, alternative final models were built by re-estimating the final models after excluding herds with an ELISA-doubtful and qPCR-negative status.

3. Results

3.1. Descriptive statistics

3.1.1. Dairy cattle

A total of 109 dairy cattle farmers were invited to participate in the study, of which 78 agreed. Among the non-participating farms, 11 had ceased operations, 18 refused to participate, with no specific reason mentioned, and two could not be reached by the research team. Of the 78 dairy cattle herds included in the study, 52 (67 %) were located in the Montérégie and 26 (33 %) in the Bas-St-Laurent regions. A total of 58 herds (74 %) were composed of Holstein cows only, and two herds (3 %) were composed of Ayrshire cows only. The other 18 herds (23 %) were mainly composed of Holstein with a few cows from one or more other breeds (Canadian, Jersey, Swiss). The number of cows in lactation per herd ranged from 22 to 200 (median = 50). Regarding cows in lactation, 67 herds (86 %) used a tie-stall system exclusively, whereas eight (10 %) used a free stall and three (4 %) used both. Various mechanical and/or natural ventilation systems were used on the farms, depending on areas in the barn and seasons.

The sampling for BTM was done from May 30 to October 14, 2011. A total of 223 milk samples were submitted to the laboratory; three BTM samples were submitted for 71 herds, but due to some logistic issues only two and one BTM samples were obtained for three and four herds, respectively. All milk samples were analyzed by qPCR, while all but four were tested by ELISA (Supplementary Fig. S1). The questionnaire was completed for all participating farms.

Of these 78 participating farms, feces were also collected from 155 cows in 31 randomly selected herds for qPCR assays from May 30 to August 12, 2011. Sampled cows were between 2 and 13 years old (median = 4). For the 150 cows with available information, 145 were in lactation (between 3 to 530 days in milk, with a median =150), and 61 (39 %) had a pregnancy detected.

3.1.2. Small ruminants

From the list of 51 small ruminant farms located in the two studied regions, eight had ceased operations, ten refused to participate, with no specific reason mentioned, and three were not reached by the research team. A total of 30 small ruminant herds (24 meat sheep, four dairy goat, and two meat goat herds) were fist included in the study, but one sheep herd was then excluded as 12 of the 15 sampled animals were not born on farm. Of the resulting 29 herds, 8 (28 %) were in the Montérégie region and 21 (72 %) were in the Bas-St-Laurent region. Herd sizes ranged from 57 to 1350 (median = 160) in sheep, and from 18 to 450 (median = 183) in goats, respectively. More than one animal breed was present on 26 farms (90 %) herds.

Sera and feces were collected from June 6 to October 31, 2011 from 15 different animals in each herd, except for three herd in which only seven to 11 animals could be sampled due to a small herd size, for a total of 416 animals (341 sheep, 75 goats). Sampled animals belonged to 16 different breeds and their crosses. The most frequent purebred animals were Alpine, Boer and Saanen in goats, and Rideau Arcott, Romanov, Suffolk and Polypay in sheep.

All serum samples were analyzed by ELISA and all feces samples were analyzed by qPCR except for one missing fecal sample (Supplementary Fig. S2). The questionnaire was completed for all participating farms.

3.2. Apparent prevalences

3.2.1. Dairy cattle

The prevalence of ELISA-positive and qPCR-positive BTM samples were estimated to 35.1 % and 8.5 %, respectively (Table 1). The estimated *C. burnetii* load on qPCR-positive BTM samples ranged from 200 to 5,120 gene copies/mL of milk, with a mean of 2.8 on the log scale. All fecal samples were classified as *C. burnetii*-negative by qPCR. The apparent prevalence of *C. burnetii*-positive herds was estimated to 43.2 % according to ELISA and to 21.6 % according to qPCR. The distribution of S/P ratio from BTM samples according to qPCR status and herd status is illustrated in Supplementary Figs. S3-S4.

3.2.2. Small ruminants

The prevalence of ELISA-positive animal was estimated to 33.3 % in sheep and to 49.2 % in goats, whereas the prevalence of ELISA-positive herds was estimated to 69.6 % and 66.7 % in sheep and goat herds, respectively. None of the goat and only 4.4 % of the sheep were qPCR-positive; the latter were from three different herds. In *C. burnetii*-positive herds, the proportion of *C. burnetii*-positive animal ranged from 6.7 to 86.7 % (median = 40.0 %). The distribution of S/P ratio from serum samples according to qPCR fecal status and herd status is illustrated in Supplementary Figs. S5-S6.

3.3. Spatial cluster

The geographical distribution of farms according to their *C. burnetii* status is presented in Fig. 1. No spatial cluster of *C. burnetii*-positive herd was detected (all $P \geq 0.09$).

3.4. Risk factors analysis

3.4.1. Animal level

In small ruminants, only the variable “parity” was statistically significant ($P < 0.01$). Higher odds of positivity were observed in animals that have lambed or kidded at least once compared to others (Table 2). Similar estimates and conclusions were obtained from the alternative model excluding animals with doubtful results (Supplementary Table S1)

3.4.2. Herd level

For dairy cattle herds, six variables were selected for multivariable analyses from the 23 screened variables (Table 3). The two variables “distance to the closest positive herd” and “distance to the closest dairy cattle positive herd” were highly correlated ($P < 0.001$, chi-square test). The latter was the only one retained considering that the association seems to be mostly driven by the proximity to dairy cattle positive herds since the proximity to small ruminant herds was not statistically significant. All variables were categorized, except for the “distance to the closest positive dairy cattle herd” variable which satisfied the linearity assumption. Three variables were statistically significant in the final multivariable model (Table 4). Higher odds of *C. burnetii* positivity were observed in herds for which the regular calving area was group pens compared to tie-stalls (OR = 20.6), and in herds located in an area with 1-6 small ruminant herd density per km² compared to an absence of small ruminant herds in a 5 km radius of the herd

(OR = 4.1). Finally, the odds of positivity decreased by 0.8 per kilometer of distance from the closest positive dairy or beef cattle herd.

For small ruminant herds, a total of 24 potential risk factors were screened using univariable logistic regression, of which seven were selected (all $P < 0.20$) for multivariate modeling (Table 5). The two variables "number of animals inside herd" and "number of animals with at least one full-term gestation" were correlated ($P < 0.001$); and only the second was kept for the multivariable analysis. Herds with more than 100 animals with at least one full-term gestation completed in their lifetime or with a dog on the farm had higher odds of *C. burnetii* positivity (Table 6).

For dairy cattle and small ruminant herds, the alternative models excluding herds with doubtful status led to similar estimates and conclusions (Supplementary Tables S2 and S3).

4. Discussion

Our study enlarges our knowledge on *C. burnetii* infection in dairy cattle and small ruminant herds in two agricultural regions of Québec. The participating farms were randomly selected among all registered herds in the two regions. The representativeness of our results for the studied areas is supported by the high participation percentage of 81 % (78/96) for dairy cattle farms and 73 % (29/40) for small ruminant farms. Moreover, the recruited farms represented approximately 28 % of dairy cattle and 75 % of small ruminant registered farms with ≥ 15 animals in the two studied regions. Although the study was conducted some years ago, the current situation is likely similar, considering that the epidemiological situation in the two regions prevailing at the time of the study remains stable in the subsequent years based on the reported incidence of Q fever in humans (Ayres Hutter et al., 2020) and number of cases of *C. burnetii* abortion in ruminants diagnosed by necropsy in provincial laboratories (Dre Anne Leboeuf, MAPAQ, personal communication, 2021).

4.1. Apparent prevalence

Our 47.3% apparent prevalence estimate for *C. burnetii*-positive dairy cattle herds, based on ELISA and qPCR, is close to the 39.6 % seroprevalence previously found in Québec (McKiel, 1964). Another study in the neighboring province of Ontario showed a higher cattle herd-level seroprevalence of 67 % (Lang, 1988), in which mostly dairy but also cow-calves herds were included. Our 69.6 % apparent prevalence estimate for sheep herds is lower than the 89 % found earlier in the Bas-St-Laurent region by Dolcé et al. (2003), but higher than the 21.4 % found earlier in Ontario (Lang et al., 1991). In goats, our 66.7 % herd-level prevalence is comparable to the 63.2 % found recently in Ontario (Meadows et al., 2015). In a review on *C. burnetii* infection in domestic ruminants based on 69 publications from several countries located on five continents, the apparent median prevalence *C. burnetii*-positive herd was of 37.7 % in cattle, 26.0 % in goat and 25.0 % in sheep herds (Guatteo et al., 2011). However, it is difficult to disentangle regional variations in prevalences from differences due to study designs and diagnostic methods, considering the large variations in sensitivity and specificity of the various testing approaches. Nevertheless, the level of *C. burnetii* positivity found in our study is comparable with previous results obtained in neighboring areas and other countries.

We only reported apparent prevalence, as no data was available on the sensitivity and specificity of the diagnostic tests we used in the context of a prevalence study. An excellent sensitivity of the ELISA kit has been reported in serum from cows that had aborted and were confirmed to be infected with *C. burnetii* (ID.vet Innovative Diagnostics, 2011). However, in the context of a prevalence study, the recommended cut-off might be too high as lower antibody levels are expected. According to a seroprevalence study conducted in carnivores, which was based on the same ELISA kit we used with adaptations, the optimal S/P ratio threshold for positivity was determined to 16.3 % based on a bi-model latent class mixture model (Meredith et al., 2014). This choice is coherent with our observations in small ruminant herds where the large majority of animals in ELISA- and qPCR-negative herds had a S/P ratio below 20 (Figure S6). Therefore, the cut-off used might result in an underestimation of previous infection with the bacteria. Interestingly, another study using Bayesian estimation reported no difference in sensitivity and specificity estimates of a *C. burnetii* ELISA performed on bovine blood or milk when doubtful results were classified as positive or negative (Paul et al., 2013).

In dairy cows, *C. burnetii* was only detected by qPCR in the BTM samples and not in fecal samples. Although a study reported a similar probability of *C. burnetii* shedding in milk and feces in cows (Guatteo et al., 2006), others observed an absence or lower prevalence of fecal shedding compared to milk shedding in infected cows (Guatteo et al., 2007b; Rodolakis et al., 2007). According to BTM samples, the prevalence of qPCR-positive herds was twice lower than the prevalence of ELISA-positive herds, similarly to what was previously reported in dairy cattle (Muskens et al., 2011; Anastacio et al., 2016). Only one study reported higher apparent prevalence of BTM positivity based on qPCR compared to ELISA (Angen et al., 2011). In this latter study, contrarily to ours, the DNA tested was extracted from the cream fraction layer instead of the full milk, which was reported to increase sensitivity and might partly explain the difference (Rodolakis et al., 2007). A 98 % sensitivity was previously reported for ELISA in BTM samples when the within-herd seroprevalence is at least 10 % (Muskens et al., 2011). In our study, we used repeated sampling to increase the likelihood of detecting ELISA or qPCR-positive herds with low within-herd prevalence; however, further studies are required to allow the estimation of the sensitivity and specificity of this approach.

The mean titers observed in BTM in our study (2.8 on a log scale) are similar to the ones previously reported (2.3 on a log scale) by Guatteo et al. (2007a). Titers of *C. burnetii* in dairy cattle BTM samples are known to be associated with the within-herd prevalence of shedder cows (Guatteo et al., 2007a; Czaplicki et al., 2012).

In small ruminants, we observed a discrepancy between fecal shedding and presence of antibodies in the same individuals, in agreement with other studies on domestic ruminants (Berri et al., 2001; Rousset et al., 2009; Muskens et al., 2011). According to previous studies, infectious sheep appear to shed the bacteria mainly through vaginal mucus and feces (Berri et al., 2001; Rodolakis et al., 2007; Astobiza et al., 2010). However, shedding of the bacteria generally follows an intermittent or sporadic pattern. Conversely, goats do not exhibit specific shedding pattern route (Arricau-Bouvery et al., 2003; Rodolakis et al., 2007; Astobiza et al., 2010). Nevertheless, shedding of the bacteria appears to be more frequent after parturition, even in non-abortive events, especially for small ruminants (Berri et al., 2001; Roest et al., 2012). In our study, most samples were collected from animals at the end of their lactation or during the dry period, which could have reduced the likelihood of detecting bacterial shedding.

4.2. Spatial cluster detection

Our study did not reveal any spatial cluster of positive farms. This could be associated with the presence of factors that favor the dispersal of the bacteria at a larger distance scale, such as animal movements between farms (Nusinovici et al., 2013) or introduction of the bacteria through contaminated fomites or people in the absence of strict biosecurity practices (Agger et al., 2013). This could also be related to the fact that the infection has been introduced long ago in the two areas and is now widespread.

4.3. Animal level risk factors

We used parity as a proxy variable for age in small ruminants since the birth date was not readily available. We observed an increase in *C. burnetii* positivity with parity. Given that animal positivity in our study was mostly driven by antibody positivity to ELISA, our findings is in agreement with the previously reported increase in small ruminant seropositivity to *C. burnetii* with age (Schimmer et al., 2011; Anastacio et al., 2013). These findings are consistent with an increase in environmental exposure to *C. burnetii* around or after the first lambing or kidding, when lactating females are grouped in pens, followed by a potential increase of seropositive animals over time due to recurrent exposure and/or persistence of antibodies (Joulie et al., 2017). However, a Turkish study on ovine herds reported a higher seropositivity in primiparous when compared to biparous ewes, suggesting the infection mostly occur at a young age (Kennerman et al., 2010).We did not find an association between *C. burnetii* and days in milk, as opposed to studies conducted in dairy cattle in which higher odds of *C. burnetii* seropositivity or excretion observed in cows when they were more advanced in their days in milk (Barlow et al., 2008; Paul et al., 2012). A recent longitudinal study conducted on

9 ewe lambs reported a slight decrease in serum antibody levels just before lambing (Joulie et al., 2017).

4.4. Herd level risk factors

In dairy cattle, we identified the regular use of a group pen for calving as a risk factor for herd positivity. Group pens are potential high-risk areas for transmission of the bacteria between cows, given the high level of shedding that can occur at time of parturition, the high environmental resistance of the bacteria and the challenges associated with the disinfection of group pens. The use of maternity or calving pens was also previously reported as a risk factor for *C. burnetii* antibody positivity in dairy cattle (Paul et al., 2012; Agger et al., 2013),

Proximity to the nearest *C. burnetii* positive bovine herd was associated with increased odds of positivity in dairy cattle. In this species, herds located downwind of qPCR-positive herds with high bacterial load were previously reported at higher risk of infection in presence of high wind speed (Nusinovici et al., 2017). In our study, we used the detection of antibodies and/or bacterial DNA to define a positive herd, assuming that herds with antibodies were at risk of bacterial shedding in the past. Proximity to the nearest *C. burnetii* small ruminant farm was not identified as a risk factor, but it should be noted that five times more dairy cattle herds were present in the study area compared to small ruminant herds, which could have blurred potential associations. We also observed an increase in dairy cattle positivity in areas with small ruminant herd density of 1-6 per km^2 in a radius of 5 km compared to areas with no small ruminant production. In dairy goats, proximity to the nearest goat herds with PCR-positive BTM was previously reported as a risk factor of positivity to *C. burnetii* (Schimmer et al., 2011). Schimmer et al. (2011) observed that an animal density over 25 goats per 5 km^2 increased the risk *C. burnetii* positivity on dairy goat herds (OR = 2.8). These associations, combined with the previous report of *C. burnetii* DNA content in air collected around the surroundings of infected farms (de Bruin et al., 2012), support the aerosol transmission of *C. burnetii* between ruminant farms. Additional factors, such as wind velocity, open landscape and low precipitation also appear to contribute to the aerosol dissemination of the bacteria in the environment (Tissot-Dupont et al., 2004; Schimmer et al., 2010; van der Hoek et al., 2011; Nusinovici et al., 2015). Nevertheless, it is also possible that bacterial dissemination at the local scale is done through other vectors, such as small rodents (Thompson et al., 2012; Abdel-Moein and Hamza, 2018), or people in absence of strict biosecurity practices (Agger et al., 2013). Interestingly, in a systematic review of Q fever outbreaks in humans, infective sheep or goats, but not cattle, were the likely source of infection (Clark and Soares Magalhaes, 2018). As previously hypothesized, the synchronicity in lambing or kidding, larger herd sizes, difference in management practices and increased risks of *C. burnetii* abortions in small ruminants could be involved (Clark and Soares Magalhaes, 2018). However, as supported with a recent study, cattle could represent a significant source of the infection for sporadic cases of Q fever in endemic areas (Pouquet et al., 2020). Contrary to dairy cattle herds, we did not find significant association between the positivity in small ruminant herds and the proximity to a positive herd nor to animal density, perhaps due to the limited sample size.

In this study, the number of animals with at least one full-term gestation completed (i.e. at least one kidding or lambing), an indicator of the herd size, was positively associated to *C. burnetii* positivity for small ruminant herds. This association was not observed in cows, perhaps due to the higher homogeneity in herd sizes. The number of animals on domestic ruminant farms was frequently reported as a risk factor of farm positivity to *C. burnetii* in small ruminants (Kennerman et al., 2010; Schimmer et al., 2011; Anastacio et al., 2013; Meadows et al., 2015) and cattle (McCaughey et al., 2010). Many authors hypothesized that a larger herd size, which is possibly related to a more intensive production and thus a higher animal density, increases the risk of transmission among animals. This could result in an increased risk of persistence of the bacteria in the herd once introduced, or in higher within-farm prevalence, which would increase the chances to detect the bacteria at herd level.

The presence of dogs was positively associated with *C. burnetii* positivity on small ruminant farms. In dairy cattle, a positive association was also observed in univariable analyses, but was not statistically significant ($P = 0.11$). In the Netherlands, Schimmer et al. (2011) also reported a link between the seropositivity of dairy goat herds and the presence of a dog (OR = 3.8) on the farm, whereas Cantas et al. (2011) noted an association between *C. burnetii* abortion in ruminants and the presence of carnivore species on the farm (OR = 3.3). Dog is a potential reservoir of *C. burnetii* (Willeberg et al., 1980; Boni et al., 1998; Shapiro et al., 2016). Moreover, dogs living near a ruminant farm were reported at higher risk of *C. burnetii*, and one such dog was identified as the source of a Q fever outbreak in humans in Canada (Buhariwalla et al., 1996; Boni et al., 1998), supporting their potential role in the transmission of the infection for both animals and humans.

4.5. Study limits

Due to the cross-sectional nature of our study, it was not possible to determine whether the risk factors observed in prevalent cases were associated with the introduction or the duration of the infection, or to assess the temporality of the associations. Also, the imperfect sensitivity or specificity of the diagnostic tests used, which might include the presence of PCR inhibitors in fecal samples, could have biased prevalence and risk factors estimates. However, results from our risk factor analyses seem relatively robust to this misclassification as the exclusion of animals or herds with doubtful status had no significant influence on final odds ratio estimates. Also, as we did not have the *C. burnetii* status of all farms in the studied areas, a misclassification of the exposure variables related to the distance to the nearest positive farms could have occurred. Because of the exploratory nature of this study, many potential risk factors were evaluated, which increased the likelihood of detecting a statistically significant association only by chance. Finally, the sheep and goat results were combined for the risk factor analysis due to sample size limitations and because they shared many similar risk factors of *C. burnetii* positivity based on the scientific literature. This, however, precluded the identification of potential species-specific risk factors.

5. Conclusion

Exposure to *C. burnetii* was very common in ruminant farms in Québec, with apparent prevalence estimated to 47.3 % in dairy cattle, 70.8 % in sheep and 66.7 % in goat herds. The odds of *C. burnetii* positivity for dairy cattle herds were associated with the use of group pen for calving, to the distance to the closest positive bovine herd, and to small ruminant herd density in a 5 km radius. In small ruminants, higher parity was associated with *C. burnetii* positivity at the animal level, whereas a larger herd size and the presence of a dog on the farm were associated with herd positivity. This study showed that the infection is frequent on domestic ruminant farms from the two regions studied and that some farm and animal characteristics might influence the transmission dynamics of the infection.

Ethics approval

The study protocol was approved by the Université de Montréal's Institutional Animal Ethics Committee (certificate #11-Rech-1596).

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Conflicts of interest:

The authors declare no conflict of interest.

References

Abdel-Moein, K.A., Hamza, D.A., 2018. Rat as an overlooked reservoir for *Coxiella burnetii*: A public health implication. Comp. Immunol. Microbiol. Infect. Dis. 61, 30-33. https://doi.org/10.1016/j.cimid.2018.11.002

Agerholm, J.S., 2013. *Coxiella burnetii* associated reproductive disorders in domestic animals - a critical review. Acta Vet. Scand. 55, 13. https://doi.org/10.1186/1751-0147-55-13

Agger, J.F., Paul, S., Christoffersen, A.B., Agerholm, J.S., 2013. Risk factors for *Coxiella burnetii* antibodies in bulk tank milk from Danish dairy herds. Acta Vet. Scand. 55, 80. https://doi.org/10.1186/1751-0147-55-80

Anastacio, S., Carolino, N., Sidi-Boumedine, K., da Silva, G.J., 2016. Q fever dairy herd status determination based on serological and molecular analysis of bulk tank milk. Transbound. Emerg. Dis. 63, e293-300. https://doi.org/10.1111/tbed.12275

Anastacio, S., Tavares, N., Carolino, N., Sidi-Boumedine, K., da Silva, G.J., 2013. Serological evidence of exposure to *Coxiella burnetii* in sheep and goats in central Portugal. Vet. Microbiol. 167, 500-505. https://doi.org/10.1016/j.vetmic.2013.08.004

Angen, O., Stahl, M., Agerholm, J.S., Christoffersen, A.B., Agger, J.F., 2011. Dynamics of relationship between the presence of *Coxiella burnetii* DNA, antibodies, and intrinsic variables in cow milk and bulk tank milk from Danish dairy cattle. J. Dairy Sci. 94, 5750-5759. https://doi.org/10.3168/jds.2011-4197

Arricau-Bouvery, N., Souriau, A., Lechopier, P., Rodolakis, A., 2003. Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes. Vet. Res. 34, 423-433. https://doi.org/10.1051/vetres:2003017

Astobiza, I., Barandika, J.F., Hurtado, A., Juste, R.A., García-Pérez, A.L., 2010. Kinetics of *Coxiella burnetii* excretion in a commercial dairy sheep flock after treatment with oxytetracycline. Vet. J. 184, 172-175. https://doi.org/10.1016/j.tvjl.2009.01.017

Ayres Hutter, J., Arsenault, J., Bekal, S., Brassard, J., Gariépy, C., Gaulin, C., Huot, C., Irace-Cima, A., Lowe, A.-M., Ramsay, D., Thivierge, K., T., T., 2020. Portrait des zoonoses entériques au Québec, 2000-2017 [Overview of enteric zoonoses in Quebec, 2000-2017]. Expert group on enteric diseases, Directorate on biological risks and occupational health, Institut national de santé publique du Québec Québec, Canada.

Barlow, J., Rauch, B., Welcome, F., Kim, S.G., Dubovi, E., Schukken, Y., 2008. Association between *Coxiella burnetii* shedding in milk and subclinical mastitis in dairy cattle. Vet. Res. 39, 1. https://doi.org/10.1051/vetres:2007060

Berri, M., Souriau, A., Crosby, M., Crochet, D., Lechopier, P., Rodolakis, A., 2001. Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. Vet. Rec. 148, 502-505. https://doi.org/10.1136/vr.148.16.502

Boni, M., Davoust, B., Tissot-Dupont, H., Raoult, D., 1998. Survey of seroprevalence of Q fever in dogs in the southeast of France, French Guyana, Martinique, Senegal and the Ivory Coast. Vet. Microbiol. 64, 1-5. https://doi.org/10.1016/S0378-1135(98)00247-8

Buhariwalla, F., Cann, B., Marrie, T.J., 1996. A dog-related outbreak of Q fever. Clin. Infect. Dis. 23, 753-755.

Cantas, H., Muwonge, A., Sareyyupoglu, B., Yardimci, H., Skjerve, E., 2011. Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. BMC Vet. Res. 7, 13. https://doi.org/10.1186/1746-6148-7-13

Clark, N.J., Soares Magalhaes, R.J., 2018. Airborne geographical dispersal of Q fever from livestock holdings to human communities: a systematic review and critical appraisal of evidence. BMC Infect Dis 18, 218. https://doi.org/10.1186/s12879-018-3135-4

Czaplicki, G., Houtain, J.Y., Mullender, C., Porter, S.R., Humblet, M.F., Manteca, C., Saegerman, C., 2012. Apparent prevalence of antibodies to *Coxiella burnetii* (Q fever) in bulk tank milk from dairy herds in southern Belgium. Vet. J. 192, 529-531. https://doi.org/10.1016/j.tvjl.2011.08.033

de Bruin, A., van der Plaats, R.Q., de Heer, L., Paauwe, R., Schimmer, B., Vellema, P., van Rotterdam, B.J., van Duynhoven, Y.T., 2012. Detection of *Coxiella burnetii* DNA on small-ruminant farms during a Q fever outbreak in the Netherlands. Appl. Environ. Microbiol. 78, 1652-1657. https://doi.org/10.1128/aem.07323-11

Dohoo, I.R., Martin, W., Stryhn, H.E., 2009. Veterinary epidemiologic research. VER, Inc. Charlottetown, P.E.I.

Dolcé, P., Bélanger, M.J., Tumanowicz, K., Gauthier, C.P., Jutras, P., Massé, R., Montpetit, C., Bernatchez, H., McColl, D., Artsob, H., 2003. *Coxiella burnetii* seroprevalence of shepherds and their flocks in the lower Saint-Lawrence River region of Quebec, Canada. Can. J. Infect. Dis. 14, 97-102.

Eldin, C., Mélenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., Mege, J.-L., Maurin, M., Raoult, D., 2017. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin. Microbiol. Rev. 30, 115-190. https://doi.org/10.1128/CMR.00045-16

Guatteo, R., Beaudeau, F., Berri, M., Rodolakis, A., Joly, A., Seegers, H., 2006. Shedding routes of *Coxiella burnetii* in dairy cows: implications for detection and control. Vet. Res. 37, 827-833. https://doi.org/10.1051/vetres:2006038

Guatteo, R., Beaudeau, F., Joly, A., Seegers, H., 2007a. Assessing the within-herd prevalence of *Coxiella burnetii* milk-shedder cows using a real-time PCR applied to bulk tank milk. Zoonoses Public Health 54, 191-194. https://doi.org/10.1111/j.1863-2378.2007.01043.x

Guatteo, R., Beaudeau, F., Joly, A., Seegers, H., 2007b. *Coxiella burnetii* shedding by dairy cows. Vet. Res. 38, 849-860. https://doi.org/10.1051/vetres:2007038

Guatteo, R., Seegers, H., Taurel, A.F., Joly, A., Beaudeau, F., 2011. Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. Vet. Microbiol. 149, 1-16. https://doi.org/10.1016/j.vetmic.2010.10.007

Hatchette, T., Campbell, N., Hudson, R., Raoult, D., Marrie, T.J., 2003. Natural history of Q fever in goats. Vector Borne Zoonotic Dis. 3, 11-15. https://doi.org/10.1089/153036603765627415

ID.vet Innovative Diagnostics, 2011. ID Screen® Q Fever Indirect Multi-species - Internal validation report.

Joulie, A., Rousset, E., Gasqui, P., Lepetitcolin, E., Leblond, A., Sidi-Boumedine, K., Jourdain, E., 2017. *Coxiella burnetii* circulation in a naturally infected flock of sheep: Individual follow-up of antibodies in serum and milk. Appl. Environ. Microbiol. 83. https://doi.org/10.1128/AEM.00222-17

Kennerman, E., Rousset, E., Golcu, E., Dufour, P., 2010. Seroprevalence of Q fever (coxiellosis) in sheep from the Southern Marmara Region, Turkey. Comp. Immunol. Microbiol. Infect. Dis. 33, 37-45. https://doi.org/10.1016/j.cimid.2008.07.007

Klee, S.R., Tyczka, J., Ellerbrok, H., Franz, T., Linke, S., Baljer, G., Appel, B., 2006. Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. BMC Microbiol. 6, 2. https://doi.org/10.1186/1471-2180-6-2

Kulldorff, M., 1997. A spatial scan statistic. Commun. Stat. Theory Methods 26, 1481-1496. https://doi.org/10.1080/03610929708831995

Lang, G.H., 1988. Serosurvey on the occurrence of *Coxiella burnetii* in Ontario cattle. Can. J. Public Health 79, 56-59.

Lang, G.H., Waltner-Toews, D., Menzies, P., 1991. The seroprevalence of coxiellosis (Q fever) in Ontario sheep flocks. Can. J. Vet. Res. 55, 139–142.

Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12, 518-553. https://doi.org/10.1128/CMR.12.4.518

McCaughey, C., Murray, L.J., McKenna, J.P., Menzies, F.D., McCullough, S.J., O'Neill, H.J., Wyatt, D.E., Cardwell, C.R., Coyle, P.V., 2010. *Coxiella burnetii* (Q fever) seroprevalence in cattle. Epidemiol. Infect. 138, 21-27. https://doi.org/10.1017/S0950268809002854

McKiel, J.A., 1964. Q fever in Canada. Canadian Medical Association journal 91, 573-577.

Meadows, S., Jones-Bitton, A., McEwen, S., Jansen, J., Menzies, P., 2015. *Coxiella burnetii* seropositivity and associated risk factors in sheep in Ontario, Canada. Prev. Vet. Med. 122, 129-134. https://doi.org/10.1016/j.prevetmed.2015.07.007

Meredith, A.L., Cleaveland, S.C., Denwood, M.J., Brown, J.K., Shaw, D.J., 2014. *Coxiella burnetii* (Q-Fever) seroprevalence in prey and predators in the United Kingdom: Evaluation of infection in wild rodents, foxes and domestic cats using a modified ELISA. Transbound. Emerg. Dis., 1-11.

Muskens, J., van Engelen, E., van Maanen, C., Bartels, C., Lam, T.J.G.M., 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. https://doi.org/10.1136/vr.c6106

Nusinovici, S., Frossling, J., Widgren, S., Beaudeau, F., Lindberg, A., 2015. Q fever infection in dairy cattle herds: increased risk with high wind speed and low precipitation. Epidemiol. Infect. 143, 3316-3326. https://doi.org/10.1017/S0950268814003926

Nusinovici, S., Hoch, T., Brahim, M.L., Joly, A., Beaudeau, F., 2017. The effect of wind on *Coxiella burnetii* transmission between cattle herds: a mechanistic approach. Transbound. Emerg. Dis. 64, 585-592. https://doi.org/10.1111/tbed.12423

Nusinovici, S., Hoch, T., Widgren, S., Joly, A., Lindberg, A., Beaudeau, F., 2013. Relative contributions of neighbourhood and animal movements to *Coxiella* *burnetii* infection in dairy cattle herds. Geospatial health 8, 471-477. https://doi.org/10.4081/gh.2014.36

Park, J.H., Chu, H., Yoo, S.J., Hwang, K.J., Lim, H.S., 2018. Serologic survey and risk factors for *Coxiella burnetii* infection among dairy cattle farmers in Korea. J Korean Med Sci 33, e245. https://doi.org/10.3346/jkms.2018.33.e245

Paul, S., Agger, J.F., Agerholm, J.S., Markussen, B., 2014. Prevalence and risk factors of *Coxiella burnetii* seropositivity in Danish beef and dairy cattle at slaughter adjusted for test uncertainty. Prev. Vet. Med. 113, 504-511. https://doi.org/10.1016/j.prevetmed.2014.01.018

Paul, S., Agger, J.F., Markussen, B., Christoffersen, A.B., Agerholm, J.S., 2012. Factors associated with *Coxiella burnetii* antibody positivity in Danish dairy cows. Prev. Vet. Med. 107, 57-64. https://doi.org/10.1016/j.prevetmed.2012.05.015

Paul, S., Toft, N., Agerholm, J.S., Christoffersen, A.B., Agger, J.F., 2013. Bayesian estimation of sensitivity and specificity of *Coxiella burnetii* antibody ELISA tests in bovine blood and milk. Prev Vet Med 109, 258-263. https://doi.org/10.1016/j.prevetmed.2012.10.007

Pouquet, M., Bareille, N., Guatteo, R., Moret, L., Beaudeau, F., 2020. *Coxiella burnetii* infection in humans: to what extent do cattle in infected areas free from small ruminants play a role? Epidemiol Infect, 1-16. https://doi.org/10.1017/S0950268820001880

Rodolakis, A., Berri, M., Hechard, C., Caudron, C., Souriau, A., Bodier, C.C., Blanchard, B., Camuset, P., Devillechaise, P., Natorp, J.C., Vadet, J.P., Arricau-Bouvery, N., 2007. Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. J. Dairy Sci. 90, 5352-5360. https://doi.org/10.3168/jds.2006-815

Roest, H.I., van Gelderen, B., Dinkla, A., Frangoulidis, D., van Zijderveld, F., Rebel, J., van Keulen, L., 2012. Q fever in pregnant goats: pathogenesis and excretion of *Coxiella burnetii*. PLoS ONE 7, e48949. https://doi.org/10.1371/journal.pone.0048949

Rousset, E., Berri, M., Durand, B., Dufour, P., Prigent, M., Delcroix, T., Touratier, A., 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. https://doi.org/10.1128/aem.00690-08

Schimmer, B., Luttikholt, S., Hautvast, J.L., Graat, E.A., Vellema, P., van Duynhoven, Y.T., 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. https://doi.org/10.1186/1746-6148-7-81

Schimmer, B., ter Schegget, R., Wegdam, M., Zuchner, L., de Bruin, A., Schneeberger, P.M., Veenstra, T., Vellema, P., van der Hoek, W., 2010. The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. BMC Infect. Dis. 10, 69. https://doi.org/10.1186/1471-2334-10-69

Schneeberger, P.M., Wintenberger, C., van der Hoek, W., Stahl, J.P., 2014. Q fever in the Netherlands - 2007-2010: What we learned from the largest outbreak ever. Med. Mal. Infect. 44, 339-353. https://doi.org/10.1016/j.medmal.2014.02.006

Shapiro, A.J., Norris, J.M., Heller, J., Brown, G., Malik, R., Bosward, K.L., 2016. Seroprevalence of *Coxiella burnetii* in Australian dogs. Zoonoses Public Health 63, 458-466. https://doi.org/10.1111/zph.12250

Thompson, M., Mykytczuk, N., Gooderham, K., Schulte-Hostedde, A., 2012. Prevalence of the bacterium *Coxiella burnetii* in wild rodents from a Canadian Natural Environment Park. Zoonoses Public Health 59, 553-560. https://doi.org/10.1111/j.1863-2378.2012.01493.x

Tissot-Dupont, H., Amadei, M.A., Nezri, M., Raoult, D., 2004. Wind in November, Q fever in December. Emerging Infect. Dis. 10, 1264-1269. https://doi.org/10.3201/eid1007.030724

van der Hoek, W., Hunink, J., Vellema, P., Droogers, P., 2011. Q fever in The Netherlands: the role of local environmental conditions. Int. J. Environ. Health Res. 21, 441-451. https://doi.org/10.1080/09603123.2011.574270

Welsh, H.H., Lennette, E.H., Abinanti, F.R., Winn, J.F., 1951. Q fever in California. IV : Occurrence of *Coxiella burnetii* in the placenta of naturally infected sheep. Public Health Rep. 66, 1473-1477. https://doi.org/10.2307/4587909

Willeberg, P., Ruppanner, R., Behymer, D.E., Haghighi, S., Kaneko, J.J., Franti, C.E., 1980. Environmental exposure to *Coxiella burnetii* : a sero-epidemiologic survey among domestic animals. Am. J. Epidemiol. 111, 437-443. https://doi.org/10.1093/oxfordjournals.aje.a112919

Woldeyohannes, S.M., Gilks, C.F., Baker, P., Perkins, N.R., Reid, S.A., 2018. Seroprevlance of *Coxiella burnetii* among abattoir and slaughterhouse workers: A meta-analysis. One Health 6, 23-28. https://doi.org/10.1016/j.onehlt.2018.09.002

Tables

Table 1

Prevalence and 95 % confidence intervals of *Coxiella burnetii* positivity for bulk tank milk (BTM) samples, animals and herds in dairy cattle, sheep and goat herds from two regions in Québec, Canada, from May to October 2011.

Species, type of sample and laboratory test	BTM sample level			Animal level			Herd level		
		Prevalence			Prevalence[b]			Prevalence	
	n	%	95 % CI[a]	n	%	95 % CI	n[c]	%	95 % CI[a]
Dairy cattle									
BTM									
ELISA	219	35.1	25.2 - 45.1				74	43.2	31.8 - 55.3
qPCR	223	8.5	4.5 - 12.6				74	21.6	12.9 - 32.7
ELISA + qPCR[d]	219	37.9	28.0 - 47.8				74	47.3	35.6 - 59.3
Feces (qPCR)				155	0.0	0.0 - 2.4	31	0.0	0.0 - 11.2
Sheep									
Serum (ELISA)				341	33.3	22.8 - 43.8	23	69.6	47.1 - 86.8
Feces (qPCR)				340	4.4	0.0 - 11.5	23	13.0	2.8 - 33.6
Both (ELISA + qPCR)[d]				341	37.2	25.3 - 49.1	23	69.6	47.1 - 86.8
Goat									
Serum (ELISA)				75	49.2	25.6 - 72.7	6	66.7	22.3 - 95.7
Feces (qPCR)				75	0.0	0.0 - 4.8	6	0.0	0.0 - 45.9
Both (ELISA + qPCR)[d]				75	49.2	25.6 - 72.7	6	66.7	22.3 - 95.7

[a] 95 % CI were adjusted for herd clustering.

[b] Prevalence estimates and 95 % CI adjusted for sampling weight and herd clustering.

[c] Only herds with at least two non-missing BTM results were included.

[d] A positive BTM sample, animal or herd was defined as having positive ELISA and/or positive qPCR result.

Table 2

Descriptive statistics and odds ratios from multi-level univariable logistic regressions modeling the positivity to *Coxiella burnetii* in small ruminants from two regions in Québec, Canada, from June to October 2011 (n = 296 animals from 20 herds[a]).

Variable & categories[b]	Number of animals	% *C. burnetii*-positive	Odds ratio		
			Estimate	95 % CI	*P*-value[c]
Parity					< 0.01
0	46	15.2	1.0		
1 - 3	140	42.9	6.6	2.1 - 20.7	
≥ 4	86	54.7	7.8	2.5 - 24.5	
Days in milk					0.35
1 - 30	33	39.4	*Not included in the final model*		
31 - 60	27	55.6			
≥ 61	62	46.7			
Not in lactation	155	40.0			
Previous outdoor access (during animal lifetime)					0.05
No	158	39.2	*Not included in the final model*		
Yes	115	46.1			

[a] Only *C. burnetii*-positive herds were included.

[b] Between 19 and 24 animals were excluded from the analyses depending on the risk factor due to missing value(s).

[c] *P*-values from univariable analyses.

Table 3

Descriptive statistics and *P*-value from univariable regressions modeling the positivity to *Coxiella burnetii* in dairy cattle herds from two regions in Québec, Canada, from May to October 2011 (n = 77 dairy cattle herds).

Variables and categories	Number of herds	% *C. burnetii*-positive	*P*-value
Region			0.69
Montérégie	51	51.0	
Bas-St-Laurent	26	46.2	
Cow breed			0.39
Ayrshire only or Holstein with a few others (Canadian ± Jersey ± Swiss)	19	57.9	
Holstein only	58	46.6	
Number of milking cows inside the herd			0.36
≤ 40	24	41.7	
41 - 65	34	47.1	
≥ 66	19	63.2	
Housing type for milking cows			0.47
Free-stall only ± tie-stall	10	60.0	
Tie-stall only	67	47.8	
Type of regular calving area used			0.09*
Tie-stall only	43	39.5	
Individual pen only	15	66.7	
Group pen only	7	85.7	
Mix	12	41.7	
Daily manure removing frequency in calving area[a]			0.14*
< 1	50	44.0	
≥ 1	24	62.5	
Type of physical separation from kidding/calving area			0.61
None	60	50.0	
Partial or mixed partial/total	13	53.8	
Total	4	25.0	
Outdoor access & area characteristics[a]			0.33
No outdoor access	38	52.6	
Outdoor access without wooden area nearby	24	37.5	
Outdoor access with wooden area nearby	13	61.5	
Farm distance to the closest wooden area (m)			0.84
< 250	23	47.8	
250 - 1000	20	55.0	

Variables and categories	Number of herds	% *C. burnetii*-positive	*P*-value
> 1000	34	47.1	
Sheep and/or goat on the farm			0.98
No	75	50.7	
Yes	2	0.0	
Dog on the farm			0.11*
No	55	43.6	
Yes	22	63.6	
Cat on the farm			0.43
No	8	62.5	
Yes	69	47.8	
Pigeon on the farm			0.54
None	18	55.6	
Yes	59	47.5	
Manure storage method			0.47
Mixed methods or others	12	58.3	
Manure pit	58	50.0	
Platform	7	28.6	
Distance to the closest positive dairy cattle herd (km)[b]			0.01*
≤ 1.9	20	60.0	
1.9 - 5.5	37	51.4	
> 5.5	20	35.0	
Distance to the closest positive small ruminant herd (km)			0.24
≤ 5	23	39.1	
> 5	54	53.7	
Distance to the closest positive herd (km)			0.05*
≤ 5	62	54.8	
> 5	15	26.7	
Bovine[c] herd density per km^2 in a 1 km radius			0.28
0	22	59.1	
1 - 40	26	53.9	
> 40	29	37.9	
Small ruminant herd density per km^2 in a 1 km radius			0.28
0	73	48.0	
> 0	4	75.0	
Ruminant herd density per km^2 in a 1 km radius			0.34
0	22	59.1	
1 - 50	29	51.7	
> 50	26	38.5	
Bovine[c] herd density per km^2 in a 5 km radius			0.56

Variables and categories	Number of herds	% *C. burnetii*-positive	*P*-value
≤ 25	42	52.4	
> 25	35	45.7	
Small ruminant herd density per km^2 in a 5 km radius			0.03*
0	20	30.0	
1 - 6	28	67.9	
> 6	29	44.8	
Ruminant herd density per km^2 in a 5 km radius			0.91
≤ 30	37	48.7	
> 30	40	50.0	

[a] Missing values from questionnaires.

[b] Descriptive statistics provided for the 1st, 2nd to 3rd and 4th quartiles of the distribution. This variable was modeled as a continuous variable.

[c] Including breeding dairy and beef cattle.

* Variable selected for multivariable modeling.

Table 4

Odds ratios from final multivariable logistic regression[a] modeling the potential risk factors for *Coxiella burnetii* positivity (-ELISA and/ or -qPCR) in dairy cattle herds from two regions in Québec, Canada, from May to October 2011 (n = 77 dairy cattle herds).

Variable & categories	Odds ratio		
	Estimate	95 % CI	*P*-value
Type of regular calving area used			
Group pen only vs. tie-stall only	20.6	1.6-267	0.02
Group pen only vs. individual pen only	6.2	0.39-99.2	0.20
Group pen only vs. mix	34.2	1.9-607	0.02
Distance to the closest positive bovine herd (per km)	0.80	0.65-0.97	0.03
Small ruminant herd density per km^2 in a 5 km radius			
1 - 6 vs. 0	4.1	1.00-16.8	0.05
> 6 vs. 0	0.97	0.24-4.0	0.97

[a] Hosmer and Lemeshow goodness-of-fit test: Chi-Square =10.3, 8 d.f., *P* = 0.25

Table 5

Descriptive statistics and *P*-values from univariable logistic regression modeling the positivity to *Coxiella burnetii* (ELISA and/or qPCR) in small ruminant herds from two regions in Québec, Canada, from June to October 2011 (n = 29 herds).

Variable & categories	Number of herds	% *C. burnetii*-positive	*P*-value[a]
Region			0.64
Montérégie	8	62.5	
Bas-St-Laurent	21	71.4	
Animal species			0.89
Caprine	6	66.7	
Ovine	23	69.6	
Type of production			0.28[a]
Meat	25	64.0	
Dairy	4	100	
Animal breed			0.73
Crossbred ± purebred	18	66.7	
Purebred only	11	72.7	
Number of animals in the herd			0.03*
≤ 100	7	28.6	
101 - 400	14	78.6	
≥ 401	8	87.5	
Number of animals with at least one full-term gestation			0.03*
≤ 100	9	33.3	
101 - 400	14	85.7	
≥ 401	6	83.3	
Type of regular lambing/kidding area used			0.45
Group pen or mixed methods	19	73.7	
Individual pen inside a group pen ± individual pen	10	60.0	
Litter adding frequency in lambing/kidding area after lambing/kidding			0.93
≤ 2	19	68.4	
> 2	10	70.0	
Yearly manure removing frequency			0.14*
1.5 - 4	24	75.0	
> 4	5	40.0	
Dog on the farm			0.05*
No	17	52.9	
Yes	12	91.7	
Cat on the farm			0.44
No	7	57.1	

Variable & categories	Number of herds	% *C. burnetii*-positive	*P*-value[a]
Yes	22	72.7	
Pigeon on the farm			0.60
None	14	64.3	
Yes	15	73.3	
Outdoor access & area characteristics			0.26
No outdoor access	9	88.9	
Outdoor access without wooden area close by	13	53.8	
Outdoor access with wooden area close by	7	71.4	
Ventilation quality in the farm			0.86
Passable	6	66.7	
Good	15	73.3	
Excellent	8	62.5	
Farm distance to the closest wooden area (m)			0.99
< 250	13	69.2	
250 - 1000	10	70.0	
> 1000	6	66.7	
Distance to the closest positive bovine herd (km)			0.60
≤ 5	14	64.3	
> 5	15	73.3	
Distance to the closest positive small ruminant herd (km)			0.36
≤ 5	19	63.2	
> 5	10	80.0	
Distance to the closest positive herd (km)			0.41
≤ 5	23	65.2	
> 5	6	83.3	
Bovine[b] herd density per km^2 in a 1 km radius			0.11*
0	13	84.6	
> 0	16	56.3	
Small ruminant herd density per km^2 in a 1 km radius			0.56
0	17	64.7	
> 0	12	75.0	
Ruminant herd density per km^2 in a 1 km radius			0.36
0	10	80.0	
> 0	19	63.2	
Bovine[b] herd density per km^2 in a 5 km radius			0.11*
≤ 15	13	84.6	
> 15	16	56.3	
Small ruminant herd density per km^2 in a 5 km radius			0.78
≤ 10	14	71.4	
> 10	15	66.7	
Ruminant herd density per km^2 in a 5 km radius			0.29
≤ 30	14	78.6	

Variable & categories	Number of herds	% *C. burnetii*-positive	*P*-value[a]
> 30	15	60.0	

[a] *P*-value from exact logistic regression.

[b] Including breeding dairy and beef cattle.

* Variable selected for multivariable modeling.

Table 6

Odds ratio from final multivariable exact logistic regression modeling the positivity to *Coxiella burnetii* (ELISA and/or qPCR) in small ruminant herds from two regions in Québec, Canada, from June to October 2011 (n = 29 herds).

Variable & categories	Odds ratio		
	Estimate	95 % CI	*P*-value
Number of animals with at least one full-term gestation			
> 100[a] vs. ≤ 100	17.1	2.8 - ∞	< 0.01
Dog on the farm			
Yes vs. No	12.5	1.9 - ∞	< 0.01

[a] Due to paucity of data, categories "101 - 400" and "> 400" were merged as they were not statistically different.

Figure Legends

Fig. 1. Geographical distribution of sampled 29 small ruminant farms and 77 dairy cattle herds according to their *Coxiella burnetii* status (positivity to ELISA and/or qPCR) in two regions, Montérégie and Bas-St-Laurent, in Québec, Canada. qPCR-positive herds in bulk tank milk are illustrated with a black dot. Samplings were done from May to October 2011. A Lambert conformal conic projection was used for mapping.