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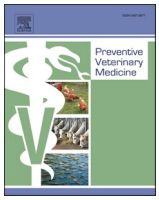

Prevalence of shedding and antibody to *Coxiella burnetii* in post-partum dairy cows and its association with reproductive tract diseases and performance: A pilot study

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ABSTRACT

The bacterium *Coxiella burnetii* has been associated with reproduction disorders in dairy cattle. A cross-sectional study was conducted in Québec, Canada, to estimate the prevalence of *C. burnetii* in dairy cows from *C. burnetii* RT-PCR-positive and/or ELISA-positive herds. As a secondary objective, the associations between *C. burnetii*-positivity and three reproductive outcomes (purulent vaginal discharge, cytological endometritis, and success at first service) were assessed. A total of 202 post-parturient dairy cows from nine herds were sampled at 35 ± 7 days in milk. Vaginal mucus and composite milk were collected from each cow and screened for the presence of *C. burnetii* by real-time PCR (RT-PCR) and ELISA, respectively. Purulent vaginal discharge and cytological endometritis were evaluated using a Metricheck device and a modified cytobrush, respectively. The first insemination postpartum was done following an ovulation synchronization protocol around 70 days in milk, and success at first service was recorded. Multilevel logistic regressions adjusted for parity were used to model purulent vaginal discharge, cytological endometritis and success at first service according to *C. burnetii* cow status. All 202 RT-PCR-assayed vaginal samples were *C. burnetii*-negative. A positive result for anti-*C. burnetii* antibodies detection in composite milk was obtained in 25/202 samples and a doubtful result in 4/202 samples. After adjustment for sampling weights, the 202 ELISA-assayed composite milk samples gave an estimated overall prevalence of *C. burnetii* positive cows of 12.9 % (CI = 6.1–19.6 %) and of doubtful cows of 1.4 % (CI = 0.0–3.3 %). The proportion of ELISA-positive cows was lower in first parity (0%) compared to second (17.1 %) or third parity cows (20.0 %). The associations between ELISA positivity and reproductive outcomes were not statistically significant, perhaps due to the limited sample size, but could be used as pilot estimate for large-scale studies investigating the impact of *C. burnetii* infection on reproduction disorders in dairy cattle.

1. Introduction

The bacterium *Coxiella burnetii* is the causative agent of Q fever (query fever), an important zoonotic disease in humans worldwide. Domestic ruminants are the main reservoir of the bacterium and can exhibit high prevalence of infection (Agerholm, 2013). In Québec, Canada, a study showed that 47.3 % (35/74) of dairy herds were positive to *C. burnetii* based on serology and RT-PCR from bulk tank milk samples (Turcotte, 2015).

There is substantial evidence of an association between *C. burnetii*

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infection in dairy cattle and sporadic abortion (Agerholm, 2013). However, *C. burnetii* infection has been inconsistently associated with metritis, endometritis, infertility and retained placenta, with some studies lacking adequate controls for evaluating the impact of the infection (López-Gatius et al., 2012; Agerholm, 2013; García-Ispierto et al., 2013; Freick et al., 2017; De Biase et al., 2018). Following the repeated findings of a negative impact of purulent vaginal discharge and cytological endometritis on dairy cattle reproduction (McDougall et al., 2011; Denis-Robichaud and Dubuc, 2015a), it becomes relevant to revisit earlier findings based on the hypothesis that optimal diagnostic criteria for these two conditions would increase the ability to detect potential associations with *C. burnetii* positivity. A better understanding of the impacts of *C. burnetii* positivity on reproductive performances in dairy cattle is essential for evaluating the cost-benefits of control measures such as vaccination programs in a farm profitability and public health perspectives.

Our study was conducted to estimate the animal-level prevalence of *C. burnetii* in dairy cows from positive herds. As a secondary objective, the associations between *C. burnetii* positivity and purulent vaginal discharge, cytological endometritis, and reproductive performance were assessed as a pilot opportunity.

2. Materials and methods

2.1. Data collection

A cross-sectional study was conducted using a convenient sample of dairy cattle herds originally recruited for a randomized clinical trial on the efficacy of intrauterine infusion of cephapirin (Denis-Robichaud and Dubuc, 2015b). Three herds with a *C. burnetii*-ELISA- or -RT-PCR-positive bulk tank milk sample established in a concurrent study (Turcotte, 2015) were first selected. To reach the target sample size of 200 cows, cows from 9 additional herds with unknown *C. burnetii* status were -ELISA- and -RT-PCR-assayed in milk and vaginal samples, respectively (see Section 2.2). Only cows from herds with at least one *C. burnetii*-ELISA- or -RT-PCR-positive sampled cows were included in the study. The sample size of 200 cows was calculated to estimate the prevalence of positive cows with a precision of 5% and a confidence level of 95 %, given an expected prevalence of 16 % (Muskens et al., 2011); we did not consider herd clustering in sample size calculation in the absence of prior data and expected limited variability in prevalence within *C. burnetii* positive farms. From selected herds, all cows that calved during the study period were sampled at 35 ± 7 days in milk. A vaginal mucus sample was first collected using a sterile BD Falcon SWUBE (Becton Dickinson, Oakville, ON, Canada). The presence of purulent vaginal discharge was evaluated with the Metricheck device (Simcro, Lawrence, KS, USA) using a cut-off ≥4 indicative of a purulent discharge or worse (Denis-Robichaud and Dubuc, 2015a). Cytological endometritis was defined as the presence of ≥6% of polymorphonuclear cells in endometrial smear collected with the modified cytobrush technique (Denis-Robichaud and Dubuc, 2015a). The evaluation of purulent vaginal discharge and cytological endometritis were performed blindly to ELISA and RT-PCR testing. A composite milk sample was aseptically collected from each cow. First insemination of all enrolled cows was synchronized around 70 days in milk using Double-Ovsynch or Presynch-Ovsynch (systematic use of the same protocol within herds). Parity number, success at first service, and herd size data were collected.

2.2. Laboratory analyses

The vaginal mucus and milk samples were kept on ice and sent to the laboratory within 12 h of collection. The vaginal mucus samples were tested by RT-PCR as described elsewhere (Klee et al., 2006). The milk samples were assayed with the ID Screen® Q Fever Indirect Multi-species ELISA kit (ID.Vet, Grabels, France) according to the manufacturer's instructions. Optical density ratio between sample and positive control (S/P) was used for interpretation: <40 % as negative, 40%–50% as doubtful, >50 % as positive.

2.3. Statistical analyses

All statistical procedures were performed in SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA) using the cow as the unit of interest. Only cows from herds with at least one cow positive to ELISA (S/P > 50 %) or RT-PCR were kept for the study. Apparent prevalence of *C. burnetii*-positive cows for RT-PCR with 95 % exact confidence intervals (CI) were estimated (due to the absence of positive sample, no adjustment for sampling design was used). Apparent prevalence of *C. burnetii* -positive or doubtful cows with 95 % confidence intervals (CI) were estimated for ELISA results, adjusted for farm clustering and sampling weights. Only apparent prevalences were reported due to the absence of validated sensitivity and specificity estimates for the diagnostic tests used (Guatteo et al., 2011). Considering the low proportion of ELISA-doubtful results, and absence of a priori information to help classify them, they were excluded from further statistical analyses. The impact of parity group on *C. burnetii* ELISA positivity was analyzed using exact chi-square, followed by post-hoc pairwise testing with Bonferroni adjustment. This approach was chosen given the presence of a zero-count cell in the contingency table for this analysis, and absence of a statistically significant association between herd and ELISA positivity in preliminary analyses (chi-square test, $P = 0.22$). Multilevel logistic regression models with random intercept for herds were used to evaluate the association between *C. burnetii* positivity and the following outcomes: purulent vaginal discharge, cytological endometritis, and success at first service as the outcome, with parity groups forced into the models as a potential confounder. Odds ratios (OR) were used to present results. Alpha value was set at 5%.

3. Results

Between August 27th and October 31st, 2012, 12 dairy herds were sampled. Three herds were excluded because no *C. burnetii*-positive cows were detected. Of the nine herds included in our study, three used a free stall housing system and six used a tie-stall barn. The herd size ranged from 65 to 220 cows (median = 90). Between nine and 70 cows (median = 20) were sampled per herd, for a total of 202 cows. Among the 196 cows with information available on parity, 62 (32 %) cows were in their 1st parity, 43 (22 %) in their 2nd and 91 (46 %) in their ≥3rd parity.

All 202 vaginal samples were *C. burnetii*-RT-PCR-negative, leading to an apparent prevalence of positive cows estimate of 0% (CI = 0.0–1.8 %) for RT-PCR. Anti-*C. burnetii* antibodies were detected in 25 milk samples and four additional samples were doubtful (Fig. 1). Thus, the estimated prevalence of ELISA-positive cows was 12.9 % (CI = 6.1–19.6 %), and was of 1.4 % (CI = 0.0–3.3 %) for ELISA-doubtful cows.

An overall association between parity group and ELISA positivity was detected ($P = 0.001$, exact chi-square), with a proportion of *C. burnetii*-ELISA positive cows of 0% in 1st parity (n = 61), 17.1 % in 2nd parity (n = 41) and 20.0 % in 3rd parity (n = 90) cows. According to post-hoc tests, 1st parity cows had a statistically significantly lower risk of positivity than 2nd or 3rd parity cows. No association with cow *C. burnetii* ELISA-positivity was observed for purulent vaginal discharge (OR) = 0.68, $P = 0.57$), cytological endometritis (OR=1.0, $P = 0.95$), or success at first service (OR=1.3, $P = 0.64$) in multilevel logistic regression models adjusted for parity groups (Table 1).

4. Discussion

The estimated apparent prevalence of *C. burnetii* ELISA-positive cows in our study was lower than the 19.4 % animal-level median apparent prevalence reported in a review conducted in cattle (Guatteo et al., 2011). In our study, only *C. burnetii*-positive herds were kept for prevalence estimation, whereas Guatteo et al. (2011) included all herds,

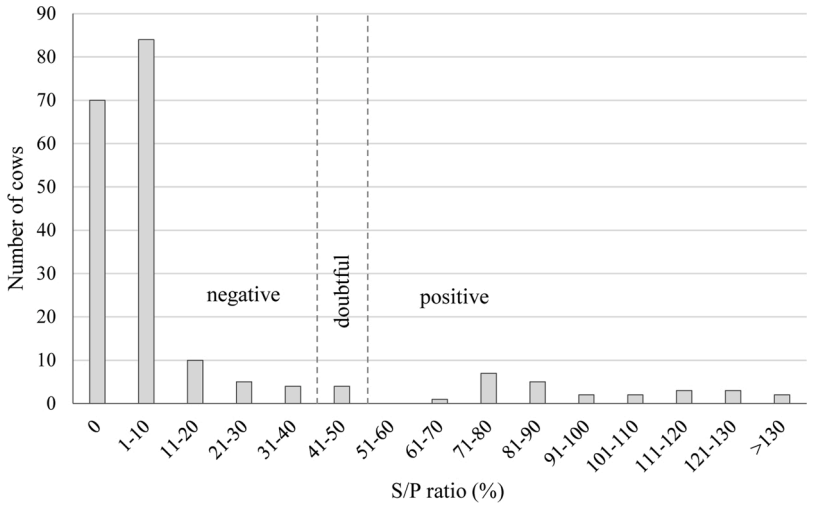

Fig. 1. Distribution of S/P ratio (%) in 202 cows from *Coxiella burnetii*-positive (ELISA and/or RT-PCR) herds, Québec, Canada, 2012.

Table 1
Multivariable analyses of the effect of *Coxiella burnetii* ELISA positivity and parity on three reproductive outcomes in dairy cows, Québec, Canada, 2012.

a. Purulent vaginal discharge (n = 191 cows[a] from 9 herds)

Variables	Number of cows	Number of cows (%) with purulent vaginal discharge	Odds ratio		
			Estimate	95 % CI	*P*-value
Coxiella burnetii ELISA status					
Positive	24	3 (12.5)	0.68	0.18−2.6	0.57
Negative	167	29 (17.4)	Ref.		
Parity					
1st	61	11 (18.0)	Ref.		
2nd	41	4 (9.8)	0.52	0.15−1.8	0.30
3rd	89	17 (19.1)	1.15	0.48−2.7	0.76

b. Cytological endometritis (n = 190 cows[b] from 9 herds)

Variables	Number of cows	Number of cows (%) with cytological endometritis	Odds ratio		
			Estimate	95 % CI	*P*-value
Coxiella burnetii ELISA status					
Positive	25	10 (40.0)	1.0	0.41−2.6	0.95
Negative	165	53 (32.1)	Ref.		
Parity					
1st	61	12 (19.7)	Ref.		
2nd	39	12 (30.8)	1.8	0.70−4.7	0.22
3rd	90	39 (43.3)	3.1	1.4−6.8	<0.01

c. Success at first service (n = 177 cows[c] from 9 herds)

Variables	Number of cows	Number of cows (%) with success at first service	Odds ratio		
			Estimate	95 % CI	*P*-value
Coxiella burnetii ELISA status					
Positive	24	9 (37.5)	1.3	0.48−3.3	0.64
Negative	153	50 (32.7)	Ref.		
Parity					
1st	60	19 (31.7)	Ref.		
2nd	38	18 (47.4)	1.9	0.79−4.4	0.16
3rd	79	22 (27.9)	0.79	0.37−1.7	0.55

[a] From the 202 cows in the study, 4 with doubtful ELISA result, 6 with missing value for parity and 1 with missing value for purulent vaginal discharge were excluded.

[b] From the 202 cows in the study, 4 with doubtful ELISA result, 6 with missing value for parity and 2 with missing value for cytological endometritis were excluded.

[c] From the 202 cows in the study, 4 with doubtful ELISA result, 6 with missing value for parity and 15 with missing value for success at first service were excluded.

which even further support the hypothesis of a low level of infection in Quebec's dairy cattle herd. On the other hand, as a decrease in antibody levels in milk samples has been reported in the weeks following calving, our prevalence might be underestimated (García-Ispierto et al., 2011; Walraph et al., 2018). The prevalence of ELISA-positive cows was very low among 1st parity cows, suggesting a lower risk of exposure before first parturition, perhaps because heifers could be kept apart from adult cows in this production system. Older cows were more likely to be ELISA-positive, as previously reported by Böttcher et al. (2011).

No significant effect of cow ELISA positivity was found on purulent vaginal discharge, endometritis, and success at first service. Despite the fact that *C. burnetii* was reported to cause inflammation in the bovine uterus and placenta, there is no strong scientific evidence supporting a negative impact of *C. burnetii* infection on metritis or endometritis in dairy cattle populations (Agerholm, 2013; De Biase et al., 2018). A recent study reported an absence of association between many parameters of reproduction and seropositivy in primiparous cows (Freick et al., 2017). A study has even observed that seropositive cows were at a lower risk of endometritis (García-Ispierto et al., 2013). Likewise, while Literak and Kroupa (1998) did not find any association between dairy cattle herd seropositivity and conception rate to first service, García-Ispierto et al. (2013) observed that *C. burnetii* shedding was associated with delayed conception among seronegative cows. On the other hand, Khalili et al. (2012) showed an association between anti-*C. burnetii* antibodies in cattle and reproductive disorders. Besides these disorders, C. *burnetii* detection by molecular method was reported in only 1% of abortion cases in dairy cattle in Quebec (Gagnon, 2013). Overall, these results suggest only minimal effects of C. *burnetii* infection on dairy cattle farm productivity. Therefore, it highlights the policy challenge of cost sharing between the industry and public health for implementing control measures on farm that would mostly benefit public health. In our study, *C. burnetii* genes had been previously detected using molecular techniques in bulk milk in two of the sampled herds (Turcotte, 2015), and the presence of ELISA-positive cows here suggests that the infection had been circulating in all herds included in our analyses. Nevertheless, the impact of the bacterial infection could depend on the state of the infection, acute or chronic, and could vary according to the animal immunity. Overall, our ELISA-positive cows had relatively low serological reactions when compared to other dairy cattle herds in which *C. burnetii* was previously identified by PCR on vaginal mucus from aborted cows (Guatteo et al., 2007). The absence of detected shedding may also imply that the bacterium was not actively circulating in the herd at time of the study. It is also possible that different *C. burnetii* strains could be involved, as previously hypothesized (Samuel et al., 1985).

The lack of association between *C. burnetii* infection and reproductive performance in our study could also be due to some design limitations. First, there may have been early culling of *C. burnetii*-infected cows with impaired reproduction performances, as previously hypothesized (García-Ispierto et al., 2011). Also, infection with *C. burnetii* could have led to endometritis, followed by clearance of the bacteria prior to sampling while the inflammation could still be detected, as previously suggested for other bacteria (McDougall et al., 2011). The cross-sectional nature of our study does not indicate the timing of this infection which would likely influence the impact on reproductive diseases. Finally, a lack of statistical power is very likely considering that the sample size was determined for another purpose. Nevertheless, this pilot study provides useful preliminary estimates for planning further larger size longitudinal studies.

5. Conclusions

At 35 days in milk, none of the 202 vaginal mucus sample was positive to *C. burnetii,* even if 25 were collected from *C. burnetii* ELISA-positive cow. Primiparous were less likely to be *C. burnetii* ELISA-positive than multiparous cows in herds known to be positive to the bacteria, suggesting the infection might occur after the first gestation. *Coxiella burnetii* ELISA positivity was not associated with reproductive disease and performance in the studied herds, but larger scale longitudinal studies are needed to confirm the absence of association. Further studies should be conducted to elucidate the herd, individual and *C. burnetii* strain-related factors associated with previously reported negative impact of this infection.

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