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John Barlow, Brad Rauch, Frank Welcome, Sung Kim, Edward Dubovi, et al.. Association between *Coxiella burnetii* shedding in milk and subclinical mastitis in dairy cattle. *Veterinary Research*, 2008, 39 (3), pp.1. 10.1051/vetres:2007060 . hal-00902909

**HAL Id: hal-00902909**

**<https://hal.science/hal-00902909>**

Submitted on 11 May 2020

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## Association between *Coxiella burnetii* shedding in milk and subclinical mastitis in dairy cattle

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(Received 20 May 2007; accepted 19 November 2007)

**Abstract** – The objective of this research was to explore the potential association between *Coxiella burnetii* shedding in milk and chronic subclinical mastitis in dairy cattle. In two separate studies, we identified an association between PCR-based detection of *C. burnetii* in milk and chronic subclinical mastitis in lactating dairy cows. These studies were conducted in a commercial dairy herd where there was ongoing intensive monitoring of subclinical mastitis by aerobic bacteriology, but no prior knowledge or management of *C. burnetii* infections. In a case-control study, quarter level *C. burnetii* status determined by real-time quantitative PCR (RT-qPCR) was strongly associated with chronic subclinical mastitis as measured by milk somatic cell counts. In a subsequent cross sectional study, 147 (45%) of 325 lactating cows were positive for *C. burnetii* by RT-qPCR of composite milk samples. In a generalized linear model, accounting for the effect of covariates including aerobic intramammary infection status, *C. burnetii* PCR status was a significant predictor of linear somatic cell count score. In agreement with a small number of previous reports, this research provides evidence that there may be mammary gland specific manifestations of *C. burnetii* infections in dairy cattle.

*Coxiella burnetii* / dairy cattle / mastitis

### 1. INTRODUCTION

*Coxiella burnetii* is an obligate intracellular zoonotic pathogen and the etiologic agent of Q fever in humans [1, 17–19]. Domestic ruminants (cattle, sheep, and goats) are described as the primary reservoir species for exposure of humans [1, 17–19]. In dairy cattle *C. burnetii* infections may be under-recognized [19, 20] and there is incomplete understanding of infection dynamics and disease manifestations. In the United States there are currently no approved *C. burnetii*

veterinary diagnostic tests or vaccines, and no state or national coxiellosis surveillance or control programs for ruminant species, although recent publications have suggested that *C. burnetii* is enzootic in US dairy herds [1, 14, 19].

*C. burnetii* infections of domestic ruminants are generally described as subclinical (i.e. asymptomatic) and persistent [1, 17–19]. Where clinical signs have been reported, they are most frequently described in sheep and goats and are related to reproductive disease [1, 2, 5, 6, 18]. Results of studies investigating the relationship between herd level seroprevalence and reproductive health in dairy cattle

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are inconsistent [8, 15, 24]. In past studies, a major limitation may have been the reliance on serology to define *C. burnetii* infection status, as serology is poor indicator of active *C. burnetii* shedding in individual animals [1, 5, 6, 9, 18]. Classically, confirmation of active infection in cattle required isolation of the organism either by laboratory animal or cell culture inoculation [1, 4, 7, 13, 16], and currently few laboratories carry out isolation due to legal limitations, risk of human exposure, and lack of sensitivity of the technique [1, 9, 18]. A small number of recent studies have described PCR-based DNA detection to identify shedding of *C. burnetii* in ruminants, including dairy cattle with reproductive disorders [5, 6, 9, 24].

While *C. burnetii* is shed for extended periods in the milk of dairy cattle, and has been shown to be immunogenic in dairy cattle, potential associations with clinical or subclinical mastitis have only rarely been examined [1, 4, 7, 13, 17, 18]. In reviews of mastitis etiologies, most authors recognize the potential public health significance of *C. burnetii* excretion in milk without describing the organism as a cause of mastitis [10, 22]. Some reviews have included *C. burnetii* among “the lesser known organisms that may cause mastitis” [21], while others did not include the organism among a broad review of bacterial etiologic agents of mastitis [25]. In 1948 and 1949 respectively, a case of chronic focal mastitis in a cow naturally infected with *C. burnetii* [13], and severe acute mastitis following experimental intramammary inoculation of dairy cattle [4] were described. A more recent report suggested that the prevalence of *C. burnetii* infections was higher among dairy cattle with reproductive problems including mastitis [24].

PCR has been described as the only veterinary diagnostic method to allow detection of specific *C. burnetii* clinical manifestations such as metritis and as the “most sensitive and rapid means to identify shedders” [1]. PCR has been used to identify shedding patterns of *C. burnetii* DNA in ruminant milk [1, 2, 6, 9], although it is currently unclear whether

shedding in milk is related to mammary specific manifestations of infection.

The objective of this work was to explore the potential association between *C. burnetii* detection in milk by RT-qPCR and chronic subclinical mastitis in a commercial dairy herd undergoing intensive mastitis monitoring by standard methods.

## 2. MATERIALS AND METHODS

### 2.1. Herd description

This study was conducted on a commercial dairy herd in north-central New York, USA. The study herd consisted of approximately 350 lactating Holstein cattle housed in a free-stall confinement barn and milked in a double-6 parallel-stall parlor. Pregnant non-lactating cows were housed in two group pens, and small groups ( $\leq 4$  cows) of periparturient cows were managed in a separate maternity pen with straw bedding cleaned regularly and replaced monthly. *C. burnetii* infection status of the herd was unknown prior to this study and there was no established surveillance or management targeting *C. burnetii* control. Sporadic and incidental metritis, infertility, and abortion events were recorded in the two years prior to this study, but no diagnostics of individual reproductive problems were completed, and no definitive causes of abortions were identified. The herd was demonstrated to be free of intramammary infections caused by *Streptococcus agalactiae* and *Mycoplasma* spp. based on extensive individual cow and bulk tank milk cultures over the proceeding 13 months.

### 2.2. *C. burnetii* PCR

Prevalence of *C. burnetii* DNA sequence in milk was assayed by RT-qPCR of quarter or composite (all quarters within a cow) milk samples collected from individual cows using previously reported methods [14]. Briefly, DNA from milk was isolated using the DNeasy Tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Isolated DNA samples were tested using primers and a probe targeting the repetitive transposon-like region (1S1111a) of the *C. burnetii* genome [12]. Real-time PCR was performed with an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Template DNA

(5 µL) was added to a reaction mixture containing 0.3 µM each primer, 0.1 µM probe, 12.5 µL of TaqMan Universal PCR Master Mix (Applied Biosystems) in a final volume of 25 µL. The cycle profile of real-time PCR was as follows: 1 cycle of 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 60 s. A standard curve was constructed using a series of 10-fold dilutions of total genomic DNA ( $10^6$  to  $10^1$  *C. burnetii* genome copies/mL) isolated from *C. burnetii* Nine Mile, phase II (RSA 493). DNase free water was used as the negative control [12].

### 2.3. Milk sampling, aerobic mastitis microbiology, and somatic cell count analysis

For three months prior to *C. burnetii* testing, monthly quarter milk samples were obtained aseptically for aerobic bacteriologic culture from all cows and held at 4 °C until processing for bacteriologic culture within 24 h. In addition, quarter milk samples from all cows at calving, dry-off, and all cases of clinical mastitis, including pre- and post-treatment were collected and held frozen at -20 °C for a maximum of two weeks until processing for bacteriologic culture. Microbiological analysis of milk samples was conducted using established methods for identification of aerobic mastitis pathogens [11,23]. Composite milk samples were obtained for determination of milk somatic cell count (SCC) by electronic counting within three days prior to monthly sampling for bacteriology.

### 2.4. Definitions

Aerobic bacteriologic status of quarters was defined based on culture results from milk taken at the time of sampling for *C. burnetii* PCR. For cows and quarters where at least three months serial culture results were available, aerobic intramammary infection (A-IMI) status was defined using definitions of Zadoks et al. [27] with a slightly more restricted definition for uninfected quarters, which were defined as a quarters with no growth of a major mastitis pathogen on three of three consecutive samples, or no growth of a minor pathogen on at least two of three consecutive samples. Major mastitis pathogens included isolation of *Staphylococcus aureus*, *Streptococcus* spp., *Arcanobacterium pyogenes*, or coliform

species, while minor mastitis pathogens were defined as coagulase negative staphylococci, or *Corynebacterium* species. For analysis of the association between *C. burnetii* status and aerobic culture results, quarter level observations for aerobic culture were merged within cow to define a cow level aerobic bacteriologic or A-IMI status.

Subclinical mastitis status was defined as an elevated milk SCC  $\geq 200\,000$  cells/mL for a single monthly observation, where no clinical event was observed in the 14 days preceding or following the SCC test date. Chronic subclinical mastitis was defined as elevated SCC for at least two consecutive months. All SCC measures were transformed to the industry standard logarithmic linear somatic cell count score (LSCS) for statistical analysis where  $LSCS = (\ln(SCC/100))/0.693147 + 3$ .

### 2.5. Study design and statistical analysis

Data were recorded in spreadsheet format (Excel, Microsoft Corp., Redmond, WA, USA), and the data file was imported to a statistical software program for subsequent data management and analysis (SAS version 9.1, SAS Institute Inc., Cary, NC, USA). Descriptive statistical analysis, including tests for normality, was completed for all variables (Proc Univariate or SAS/Insight, SAS 9.1, SAS Institute Inc.). Two separate studies were conducted.

### 2.6. Study 1

A preliminary case-control study was conducted to explore the association between subclinical mastitis and *C. burnetii* shedding among A-IMI negative cows. *C. burnetii* RT-qPCR status of quarter milk samples was compared for 39 quarter milk samples from 10 cows with culture negative chronic subclinical mastitis (SCC  $\geq 200\,000$  cells/mL and no major pathogen isolated for two consecutive months; cases) and 39 quarter milk samples from 10 cows with culture negative continuous low SCC (SCC  $< 200\,000$  cells/mL and no major pathogen isolated for two consecutive months; controls). Case and control cows were matched on lactation number and days in milk. The association between the number of *Coxiella burnetii* RT-qPCR (CbPCR) positive quarters within cows and cow level subclinical mastitis status was examined using contingency tables and Cochran-Mantel-Haenszel statistics, with significance of association set at

$p < 0.05$ . A generalized linear model was used to test for differences in the mean average somatic cell count score among cows stratified by number of CbPCR positive quarters, with significance set at  $p < 0.05$ .

## 2.7. Study 2

Data from a cross sectional study of all lactating cows in the herd was used to test for an association between *C. burnetii* shedding in milk and subclinical mastitis in a series of hierarchical linear and logistic regression models (Proc Mixed and Proc Genmod, SAS 9.1, SAS Institute Inc.) that included up to seven individual predictors and any 2-way interaction significant at the  $p \leq 0.05$  level. The full linear regression model using the three month average LSCS (AVLS) as the outcome variable was  $AVLS = \beta_0 + \beta_1 CbPCR + \beta_2 A - IMI + \beta_3 LACTGRP + \beta_4 DIMCAT + \beta_5 CLIN + \beta_6 MILK + \beta_7 ME305 + \beta_8 X_1 \times \beta_9 X_2 + \dots$ , where CbPCR is *C. burnetii* infection status as measured by RT-qPCR, A-IMI is the aerobic bacterial intramammary infection status, LACTGRP is the lactation group number, DIMCAT is the days in milk category, MILK is the pounds of milk produced at the most recent test, ME305 is the predicted 305 day mature equivalent milk production, and CLIN is clinical mastitis status, of each individual cow. A-IMI status was a categorical variable describing the pathogen specific infection status of the cow for common species and groups of mastitis pathogens (i.e. *Staphylococcus aureus*, *Streptococcus* spp., other major pathogens (OMP), coagulase negative staphylococci (CNS), other minor pathogens (OTHMINOR), and aerobic culture negative samples (NEG)). Lactation group reflects the age of the cow and was a categorical variable with three categories defined as lactation 1 (primiparous cows), lactation 2 (cows in second lactation), and lactation 3 or greater. Days in milk is the number of days currently lactating, and ten categories were defined by 30 day intervals from one day post partum to 270 days, with the final category defined as  $\geq 270$  days in milk. Clinical status was a binary categorical variable defined as 1 for cows that had a clinical mastitis event in the 14 days prior to or following milk sampling for RT-qPCR testing. Final model selection was based on goodness of fit tests including change in deviance observed for the two hierarchical models being compared, as

well as comparison of Akaike's information criteria (AIC) and Bayesian information criteria (BIC) fit statistics, with CbPCR forced into all models. The potential association between *C. burnetii* shedding in milk and subclinical mastitis was also evaluated using logistic regression models developed in the same manner, but where the dependent variable was a binary variable defining a cow as having a persistent high SCC for three of three sequential test dates prior to CbPCR testing. For all independent variables included in the final models, Pearson correlation coefficients and tests of multicollinearity were explored using the Proc Corr and Proc Reg procedures, respectively (SAS 9.1, SAS Institute Inc.).

## 3. RESULTS

### 3.1. Bacteriologic culture and somatic cell counts

Quarter milk samples were collected for aerobic bacteriologic culture from 351 cows. Three consecutive months of serial aerobic culture results were available to define A-IMI status for 222 cows, and one, two, and three months of SCC data were available for 195, 189, and 185 of these cows, respectively. At the cow level, 21 cows had IMI caused by a major pathogen, 84 cows had IMI caused by a minor pathogen, and 23 cows had quarters with IMI caused by two pathogen types (mixed IMI) being predominately *Streptococcus* spp. plus either *Corynebacterium* spp. or CNS.

### 3.2. Study 1 – case-control study results

There were 31 CbPCR positive and 8 CbPCR negative quarters among cows with culture negative chronic subclinical mastitis, and 11 CbPCR positive and 28 CbPCR negative quarters among culture negative cows with persistent low SCC. Eight cows with chronic subclinical mastitis were CbPCR positive in all four quarters, and two cows with chronic subclinical mastitis were CbPCR negative in all four quarters. In comparison, 5, 3, and 2 cows with persistent low SCC had 0, 3, and 4 CbPCR positive quarters, respectively. The number of CbPCR positive quarters within cows was positively associated

**Table I.** Number of *Coxiella burnetii* PCR (CbPCR) positive and negative cows stratified by aerobic pathogen intramammary infection (A-IMI) status, lactation group, and days in milk group.

Lactation group	Days in milk category	All cows		Cows with 3 serial monthly bacteriologic culture results defining A-IMI status					
		CbPCR positive	CbPCR negative	CbPCR positive			CbPCR negative		
				Major pathogen A-IMI	Minor pathogen A-IMI	No A-IMI	Major pathogen A-IMI	Minor pathogen A-IMI	No A-IMI
1	1-90	5	15	0	0	0	0	0	0
	91-180	12	23	0	0	0	0	0	0
	181-270	11	12	0	1	0	0	1	0
	>270	36	17	1	15	9	1	8	6
Total lactation group 1		64	67	1	16	9	1	9	6
2	1-90	3	8	0	0	0	0	0	0
	91-180	8	18	0	1	3	0	3	5
	181-270	8	8	2	4	2	1	4	3
	>270	11	13	1	4	4	2	5	4
Total lactation group 2		30	47	3	9	9	3	12	12
≥3	1-90	2	11	0	1	0	0	0	0
	91-180	9	13	0	5	3	2	2	4
	181-270	19	21	4	9	6	2	8	9
	>270	23	19	3	9	10	2	7	5
Total lactation group 3		53	64	7	24	19	6	17	18
All	1-90	10	34	0	1	0	0	0	0
	91-180	29	54	0	6	6	2	5	9
	181-270	38	41	6	14	8	3	13	12
	>270	70	49	5	28	23	5	20	15
Total		147	178	11	49	37	10	38	36

with chronic subclinical mastitis status of cows (Cochran-Mantel-Haenzel statistic  $p = 0.0236$ , on two degrees of freedom). The mean LSCS (s.d) of the 10 cows with 4 CbPCR positive quarters was 4.82 (1.95), compared to means of 1.20 (0.56) and 2.72 (2.23) for cows with 1 and 0 positive quarters respectively.

**3.3. Study 2 – cross sectional survey results**

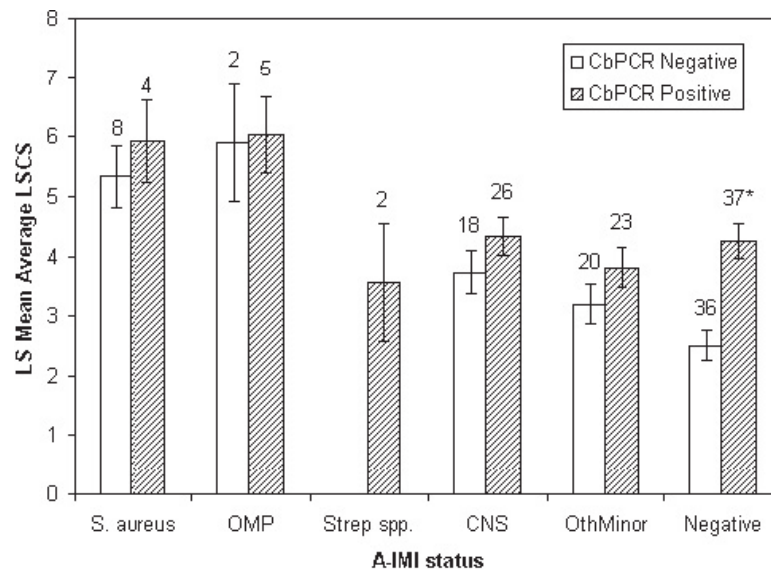
One-hundred-forty-seven (45%) of 325 cows tested were positive for *C. burnetii* and cows later in lactation at the time of testing were more likely to be found CbPCR positive (Tab. I).

*C. burnetii* PCR status was a significant predictor of AVLS in all linear models

regardless of number of covariates included. All covariates except ME305 were significant predictors of AVLS in multivariate models, and no 2-way interactions were significant or found to improve model fit. Table II includes results of the final linear regression model indicating CbPCR was a significant predictor of somatic cell count after correcting for the effect of five significant covariates. The mean somatic cell count of CbPCR positive cows was numerically higher than CbPCR negative cows regardless of infection status, but the difference was greatest among A-IMI negative cows (Fig. 1). For example, among cows with an A-IMI caused by *S. aureus* the three month average LSCS of CbPCR positive and negative cows were 5.93 and 5.33, respectively. In comparison, the least

**Table II.** Parameter estimates for the multivariate model of the association between *Coxiella burnetii* quantitative PCR (CbPCR) status and the three month average linear somatic cell count score (AVLS) in 181 lactating dairy cattle. Aerobic intramammary infection (A-IMI) categories: *Staphylococcus aureus*, *Streptococcus* spp., other major pathogens (OMP), coagulase negative Staphylococci (CNS), other minor pathogens (Minor), days in milk category (DIMCAT), lactation group number (LACTGRP), pounds of milk produced at the most recent test (MILK).

Model AVLS = CbPCR + A-IMI + CLINICAL + DIMCAT + LACTGRP + MILK					
Effect	Estimate	Standard Error	DF	T Value	Pr >  t
Intercept	5.4244	0.4384	163	12.37	<0.0001
CbPCR	1.0460	0.2058	163	5.08	<0.0001
<i>S. aureus</i>	2.1839	0.4156	163	5.25	<0.0001
Strep spp.	-0.2361	0.9608	163	-0.025	0.8062
OMP	2.1946	0.5352	163	4.10	<0.0001
CNS	0.6373	0.2572	163	2.48	0.0142
Minor	0.1535	0.2565	163	0.60	0.5503
Clinical	1.0912	0.3050	163	3.58	0.0005
DIMCAT	-	-	-	-	-
LACTGRP	-	-	-	-	-
MILK	-0.02174	0.0050	163	-4.33	<0.0001



**Figure 1.** Least square (LS) mean three month average linear somatic cell count (LSCS) score for *Coxiella burnetii* RT-qPCR (CbPCR) positive and negative cows stratified by aerobic intramammary infection (A-IMI) status where A-IMI status was based on at least three monthly serial aerobic bacteriologic culture results (error bars indicate standard error, *n* above bars = number of cows; A-IMI categories: *Staphylococcus aureus*, other major pathogens (OMP), *Streptococcus* spp., coagulase negative staphylococci (CNS), other minor pathogens (OthMinor), and aerobic culture negative samples (Negative)). \* Among cows that were defined as A-IMI negative (*n* = 73) the mean three month average LSCS of CbPCR positive cows was significantly greater (*p* = 0.002) than that of CbPCR negative cows.

squares mean three month average LSCS of A-IMI negative CbPCR positive cows was 4.25 compared to 2.51 for A-IMI negative CbPCR negative cows (Fig. 1). The median SCC calculated from the raw data for CbPCR positive and negative cows with an IMI caused by a major mastitis pathogen were 762 000 and 528 000 cells/mL, respectively, and for CbPCR positive and negative cows with an A-IMI caused by a minor mastitis pathogen were 233 000 and 203 000 cells/mL, respectively. In comparison, the median SCC of cows with no evidence of an intramammary infection caused by aerobic pathogens was 229 000 and 82 000 cells/mL, for CbPCR positive and negative cows, respectively. From the final multivariate logistic model, the odds of a chronic high cell count among CbPCR positive cows are 3.92 (95% CI, 1.63 < OR < 9.39) times that of CbPCR negative cows, after accounting for the effect of other variables. In both linear and logistic multivariate models, CbPCR status was also a significant predictor of either AVLS or chronic subclinical mastitis when aerobic bacteriologic culture status (based on a single milk culture at the time of PCR testing) was substituted for A-IMI status (data not shown). Significant correlations were found between CbPCR status and DIMCAT (correlation coefficient = 0.255,  $p < 0.001$ ), MILK and DIMCAT ( $-0.393$ ,  $p < 0.001$ ), CLIN and MILK ( $-0.145$ ,  $p = 0.009$ ), and A-IMI status and DIMCAT ( $0.138$ ,  $p = 0.049$ ); all remaining correlation coefficients were  $< 0.08$ . Further, no problem of collinearity was identified as all variance inflation factor values were  $< 1.5$ , and the variance inflation factor for CbPCR and A-IMI status was 1.04 and 1.02, respectively.

#### 4. DISCUSSION

In comparison to previous studies suggesting *C. burnetii* is associated with bovine mastitis, a major strength of our studies is that they were conducted in a herd where there was ongoing intensive monitoring of subclinical mastitis infection dynamics. A key finding was that *C. burnetii* shedding in milk was associated with chronic subclinical mastitis

in cows demonstrated to be free of infection caused by common aerobic mastitis pathogens. This is in contrast to three previous reports of mastitis associated with *C. burnetii* infection that did not appear to investigate the possibility of mastitis caused by common aerobic mammary pathogens [4, 13, 24]. Although we were able to account for the potential effect of bacterial mastitis pathogens identified by routine aerobic culture, we could not eliminate the possible role of other less common bacterial or viral pathogens that might be associated with subclinical mastitis [21, 25, 26]. Despite this limitation, our finding that A-IMI negative CbPCR positive cows had a median SCC of  $> 200\,000$  cells/mL compared to  $< 100\,000$  cells/mL for A-IMI negative CbPCR negative cows is of particular interest from the perspective of mastitis control, as SCC is used as a diagnostic screening test in mastitis control programs [23]. Further, the observation that the SCC of cows with both *C. burnetii* positive milk and A-IMI was increased relative to CbPCR negative A-IMI positive cows, suggests a possible effect of co-infection of *C. burnetii* and common mastitis pathogens, although the nature and clinical relevance of this potential interaction is unknown. Other associations observed in this study that warrant further analysis include the finding that CbPCR positive status increased with increasing days in milk. Because the history and true prevalence of *C. burnetii* infections in this herd was unknown it is unclear how our observations may have been affected by duration of *C. burnetii* infection in the herd or individual animals. Further, because the organism was not isolated and the strain type was not identified it is unknown if the mammary specific disease manifestations observed in this herd could have been influenced by the involved strain(s) [3].

These studies were limited to PCR-based detection of *C. burnetii* DNA sequence in milk samples, so we are unable to speculate on the viability of organisms in milk of cows in this commercial herd, or on the sensitivity and specificity of the PCR relative to other diagnostic methods. Current alternatives to diagnosis *C. burnetii*



infection in ruminants include serological analysis, organism isolation by either cell culture (e.g. shell vial culture) or live animal inoculation, immunohistochemical and PCR-based detection [1, 8, 17, 18]. Previous reports have demonstrated that viable organisms may be variably shed in the milk of dairy cattle for extended periods of greater than one year and across multiple lactations [1, 7, 17], and that within a herd the proportion of cows shedding in milk may range from 15 to 62% [7, 9]. Our finding that 45% of cows in this commercial herd were CbPCR positive in milk samples is consistent with these previous reports. To the best of our knowledge, only two studies have compared results of PCR detection of *C. burnetii* in milk with bacteria viability assays by mouse inoculation [2, 24]. While these two studies demonstrate PCR positive milk samples contain viable organisms, additional studies are needed to determine how PCR based detection relates to the potential infectiousness of *C. burnetii* in milk samples, or the sensitivity and specificity of PCR relative to inoculation or antigen detection assays. The ability to obtain such data is likely restricted to a relatively small number of research laboratories, as isolation must be conducted in biosafety-level 3 laboratories [1], and may be legally restricted in the United States where the organism is classified as a category B bioterrorism agent by Centers for disease Control and Prevention (CDC).

*Coxiella burnetii* infections of dairy cattle have received relatively little attention in North America [8]. Shedding of the organism from other tissue sights and other potential disease manifestations were not explored in this herd. While our studies were limited to a single commercial dairy herd and focused on mammary specific detection of *C. burnetii* to evaluate a potential association with subclinical mastitis, the finding that *C. burnetii* PCR status in milk is associated with increased SCC, an economically important milk quality measure and diagnostic tool for mastitis control, suggests that additional research is justified to evaluate the consequences of *C. burnetii* infections in dairy cattle.

**Acknowledgements.** The authors would like to acknowledge the contribution of the farm owner and farm staff and his veterinary practitioner in conducting this study. The support of our field and laboratory staff is also greatly appreciated.

## REFERENCES

- [1] Arricau-Bouvery N., Rodolakis A., Is Q fever an emerging or re-emerging zoonosis? *Vet. Res.* (2005) 36:327–349.
- [2] Arricau-Bouvery N., Souriau A., Bodier C., Dufour P., Rousset E., Rodolakis A., Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats, *Vaccine* (2005) 23:4392–4402.
- [3] Arricau-Bouvery N., Hauck Y., Bejaoui A., Frangoulidis D., Bodier C.C., Souriau A., Meyer H., Neubauer H., Rodolakis A., Vergnaud G., Molecular characterization of *Coxiella burnetii* isolates by infrequent restriction site-PCR and MLVA typing, *BMC Microbiol.* (2006) 6:38.
- [4] Bell E.J., Parker R.R., Stoenner H.G., Q fever: experimental Q fever in cattle, *Am. J. Public Health* (1949) 39:478–484.
- [5] Berri M., Crochet D., Santiago S., Rodolakis A., Spread of *Coxiella burnetii* infection in a flock of sheep after an episode of Q fever, *Vet. Rec.* (2005) 157:737–740.
- [6] Berri M., Rousset E., Hechard C., Champion J.L., Dufour P., Russo P., Rodolakis A., Progression of Q fever and *Coxiella burnetii* shedding in milk after an outbreak of enzootic abortion in a goat herd, *Vet. Rec.* (2005) 156:548–549.
- [7] Biberstein E.L., Behymer D.E., Bushnell R., Crenshaw G., Riemann H.P., Franti C.E., A survey of Q fever (*Coxiella burnetii*) in California dairy cows, *Am. J. Vet. Res.* (1974) 35:1577–1582.
- [8] Bildfell R.J., Thomson G.W., Haines D.M., McEwen B.J., Smart N., *Coxiella burnetii* infection is associated with placentitis in cases of bovine abortion, *J. Vet. Diagn. Invest.* (2000) 12:419–425.
- [9] Guatteo R., Beaudou 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) 37:827–833.
- [10] Heidrich H.J., Renk W., Inflammation of the udder, in: Heidrich H.J., Renk W. (Eds.), *Diseases of the mammary gland of domestic animals*, W.B. Saunders, Philadelphia, PA, USA, 1967, pp. 113–116.
- [11] Hogan J.S., Gonzalez R.N., Harmon R.J., Nickerson S.C., Oliver S.P., Pankey J.W., Smith K.L., *Laboratory handbook on bovine mastitis*, National Mastitis Council Inc., Verona, WI, USA, 1999.

- [12] Hoover T.A., Vodkin M.H., Williams J.C., A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence, *J. Bacteriol.* (1992) 174:5540–5548.
- [13] Jellison W.L., Ormsbee R., Beck M.D., Parker R.R., Bell E.J., Q fever studies in southern California: V. Natural infection in a dairy cow, *Public Health Rep.* (1948) 63:1611–1618.
- [14] Kim S.G., Kim E.H., Lafferty C.J., Dubovi E., *Coxiella burnetii* in bulk tank milk samples, United States, *Emerging Infect. Dis.* (2005) 11:619–621.
- [15] Literak I., Kroupa L., Herd-level *Coxiella burnetii* seroprevalence was not associated with herd-level breeding performance in Czech dairy herds, *Prev. Vet. Med.* (1998) 33:261–265.
- [16] Lorenz H., Jager C., Willems H., Baljer G., PCR detection of *Coxiella burnetii* from different clinical specimens, especially bovine milk, on the basis of DNA preparation with a silica matrix, *Appl. Environ. Microbiol.* (1998) 64:4234–4237.
- [17] Marrie T.J., Raoult D., Q fever – a review and issues for the next century, *Int. J. Antimicrob. Agents* (1997) 8:145–161.
- [18] Maurin M., Raoult D., Q fever, *Clin. Microbiol. Rev.* (1999) 12:518–553.
- [19] McQuiston J.H., Childs J.E., Q fever in humans and animals in the United States, *Vector Borne Zoonotic Dis.* (2002) 2:179–191.
- [20] McQuiston J.H., Holman R.C., McCall C.L., Childs J.E., Swerdlow D.L., Thompson H.A., National surveillance and the epidemiology of human Q fever in the United States, 1978–2004, *Am. J. Trop. Med. Hyg.* (2006) 75:36–40.
- [21] Philpot W.N., Pankey J.W., Review of microorganisms that reportedly cause mastitis, Hill Farm Research Station, Homer, LA, USA, 1975, pp. 118–120.
- [22] Rebhun W.C., *Miscellaneous infectious diseases*, in: *Diseases of dairy cattle*, Lippincott Williams and Wilkins, Philadelphia, PA, USA, 1995, pp. 481–482.
- [23] Sears P.M., Gonzalez R.N., Wilson D.J., Han H.R., Procedures for mastitis diagnosis and control, *Vet. Clin. North Am. Food Anim. Pract.* (1993) 9:445–468.
- [24] To H., Htwe K.K., Kako N., Kim H.J., Yamaguchi T., Fukushi H., Hirai K., Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders, *J. Vet. Med. Sci.* (1998) 60:859–861.
- [25] Watts J.L., Etiological agents of bovine mastitis, *Vet. Microbiol.* (1988) 16:41–66.
- [26] Wellenberg G.J., van der Poel W.H., Van Oirschot J.T., Viral infections and bovine mastitis: a review, *Vet. Microbiol.* (2002) 88:27–45.
- [27] Zadoks R.N., Allore H.G., Barkema H.W., Sampimon O.C., Grohn Y.T., Schukken Y.H., Analysis of an outbreak of *Streptococcus uberis* mastitis, *J. Dairy Sci.* (2001) 84:590–599.