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Coxiella burnetii in dairy cattle herd: farming practices and infection dynamics

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Abstract. Q fever is a widespread disease affecting reproduction in dairy cattle. Several risk factors can increase the possibility of the herd becoming infected and the persistence of infection. The aim of this study was to characterise the dynamics of *C. burnetii* infection in affected herds and to evaluate reproduction problems and risk factors using a questionnaire within infected and infection-free dairy herds. In total, 25 farms that were serologically positive or shedding *C. burnetii* DNA in milk and 14 *C. burnetii* infection-free farms in Latvia answered the questionnaire. Ten positive herds were studied by testing individual blood and milk samples from up to 10 animals at two times separated by 7.5 to 13 months. The number of serologically positive and suspicious animals was higher in the second sampling even though several animals were culled. In the positive herds, the percentage of dystocia, stillborn calves and abortions during the last year was significantly (p=0.001; 0.01; 0.005, respectively) higher than in the negative herds. Several significant factors were found for the presence of Q fever infection, such as the herd size, the regional population density of ruminants, artificial ventilation systems, and frequent farm visitors. Deratisation was less practised in the negative farms and the presence of ticks was observed more often in the negative farms. Only two identified risk factors can be mitigated – the ventilation system and frequent farm visitors. The other factors cannot be changed arbitrarily but they have to be taken into account by decision makers.

Key words: Q fever, dairy cattle, shedding in milk, serological response, farming practices

Introduction

Q fever or coxiellosis is a widespread zoonotic disease. The prevalence and incidence of coxiellosis is higher among people who work directly with infected livestock, such as farmers, veterinarians, veterinary medicine students, slaughterhouse staff, etc. (1). The disease has been reported from all over the world (2). The causative agent of Q fever is bacterium *Coxiella burnetii*. *C. burnetii* can cause infections in a wide range of hosts – mammals (including humans), birds and arthropods. *C. burnetii* is a Gram-negative obligate intracellular, pleomorphic bacterium, which can survive and even multiply in the phagolysosomes of phagocytes (3). Another specific feature of *C. burnetii* is its two antigenic stages – Phase I variant (PhI) and Phase II variant (PhII). In cattle, $PhI^{-}/PhII^{+}$ indicates an acute infection, while change to $PhI^{+}/PhII^{+}$ indicates chronic infection (4).

Within the time period of 1950-2009, high seroprevalence in cattle was observed in Europe and neighbouring countries – 0.6-44.9% in countries such as Albania, Bulgaria, Cyprus, Denmark, Italy, Germany, the Netherlands, Spain, Turkey, the United Kingdom (Northern Ireland) (5). *C. burnetii* may cause metritis, infertility, late term abortions, and stillbirths in dairy cattle (3, 7). Infected ruminants often show no clinical symptoms, including a lack of fever (6). Infected animals shed *C. burnetii* mainly through amniotic fluids and placenta, but also via vaginal mucus, milk, urine, faeces, and semen. The kinetic patterns and routes of shedding vary between cows and depend on the strain of *C. burnetii* - the shedding can be absent, persistent or sporadic

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(7-8). Parturition is considered as a period at risk; at this time, large numbers of bacteria are shed via different routes, especially amniotic fluid and the placenta (9). Shedding can persist for several months after parturition, and it can even be intermittent. In the postpartum period, bacteria are shed via vaginal mucus, milk and faeces, but none of these routes is predominant (7, 9).

C. burnetii is highly resistant to environmental factors such as temperature, desiccation, and also the most commonly used disinfectants. Appropriate biosecurity measures taken by farm workers, herd veterinarians, and visitors can significantly reduce the spread of this pathogen. Biosecurity measures that affect the transmission of disease and contamination of the environment are the prompt removal of aborted foetus and placenta; proper processing of manure (composting, disinfection, application on damp soil); deratisation; restriction of domestic and wild animal access to livestock; strict hygiene measures taken by the veterinarian before entering the farm and excluding visitors (5, 7, 10). Other risk factors associated with the presence of *C. burnetii* in herds are a free stall dairy barn system, open farm management, sharing equipment with other farms, animal transport vehicles and hoof trimmers as mechanical transmitters of infection, an increase in regional herd density and animal population, herd size and the age of animals (11-14).

If the presence of Q fever infection in the herd is detected, this infection should be considered endemic in the cattle population of the particular territory. Clinical manifestation generally subsides after the initial introduction of the pathogen in the herd, but shedding will continue for multiple years, even if a vaccination programme has been started. It is very difficult to eliminate the presence of this organism, because of its ubiquitous nature and the low infectious dose (2). It is critical to control the transmission between animals and to reduce environmental contamination, thereby minimising the zoonotic disease risk. Management of parturient animals plays a major role in the transmission of disease within herds, because animals around that time shed very high amounts of pathogen in the environment. Parturient animals should be kept in an enclosed environment with controlled airflow, the aborted foetus and placenta must be rapidly removed and the development of an excessively dry and dusty environment around the parturition area should be avoided by generating mist or moistening (5, 10).

Serology is the method of choice for diagnosis, because it is difficult to diagnose Q fever clinically. Although serology is comprehensively applied for diagnosis, some shedders remain seronegative (8), whereas positive antibody titre provides evidence of a current or earlier infection with *C. burnetii*, but is not necessarily indicative of current or prior shedding of the infectious agent (10).

There is no gold standard for precise diagnosis but different laboratory techniques are available. Currently, the most accurate diagnostic test is direct detection and quantification by PCR (polymerase chain reaction) and serological test ELISA (enzyme-linked immunosorbent assay). The PCR method is rapid and can be used on a wide range of samples (vaginal discharge, aborted tissues, faeces, and milk) (3, 6).

The aim of this study was to compare some aspects of farming practices in both *C. burnetii* affected and *C. burnetii* free dairy herds, in order to detect the dynamics of *C. burnetii* infection in the affected herds and to evaluate risk factors by using a questionnaire.

Materials and Methods

A questionnaire about farming practices

In this study, a total of 200 dairy herds in Latvia were selected for the questionnaire (Supplementary material) about reproductive problems and farming practices. The total number of dairy herds in Latvia was approximately 11,800 in December 2020 (15). Dairy herds were selected in accordance with previous records from the database at the Institute of Food Safety, Animal Health and Environment "BIOR" containing information about bulk tank milk and instances of abortion in cattle. The database included information starting from 2012, reflected in the previous study (16), and up to September 2019. One hundred of the farms were considered as Q fever negative, i.e., there were no positive records (ELISA, PCR) in the database regarding these farms – aborted tissues and bulk tank milk (BTM) samples were serologically negative, BTM samples and aborted foetuses were PCR negative. Another hundred farms were considered as positive, having at least one positive test result in the database. The selected farms represented 52 out of the 110 local regions of Latvia. The distribution of selected farms covered various parts of the country but, due to the uneven Q fever prevalence in the country (16), a larger fraction of positive farms was found in the Western-Central and Northern part, while several negative farms were located in the Eastern part of the country.

Due to the low response from farmers, the survey using the questionnaire was attempted several times within the time period of 2019-2020. The questionnaire was created in Google forms and sent out by e-mail. Those farmers not responding were contacted by phone, if possible. In 10 positive farms, the questionnaires were filled out together with the project experts while on site at the farm. Fourteen

farms answered that they do not have cattle anymore. In total, only 39 farms answered the questionnaire. Twenty-five of them were serologically positive or shedding *C. burnetii* DNA in milk (14 570 animals) and 14 other farms were negative (975 animals). Besides several risk factors that could have impact on the introduction and persistence of Q fever in the herds, several questions were included in order to characterise reproductive problems, such as the percentage of dystocia and stillborn calves during the last year, and the percentage of cattle that suffered from abortion during the last year.

Sampling and analysis

Ten of the positive herds were studied in more detail. The actual information of the year 2018 from the database of the Institute of Food Safety, Animal Health and Environment "BIOR" was used. These ten farms were registered as positive, having at least one recent positive ELISA test result after abortion or positive PCR result of the bulk tank milk sample. They were visited by the veterinarian who had been involved in the study and evaluated by testing individual blood and milk samples from up to 10 animals from each herd. The individual samples were taken two times with a 7.5 to 13 month interval, in order to analyse the dynamics of infection. The animals for the first sampling were randomly selected from both groups: pregnant heifers and lactating cows. From every herd, between two and four animals with previous reproductive problems were chosen, including cases of repeated artificial insemination (22 animals in total), previous abortions or stillborn calves (28 animals in total). Other animals without reproductive problems were selected to represent different lactations – from the first to the seventh (Table 1). This sampling scheme was based on the diagnostic and sampling approach of the Passive monitoring scheme in cattle, suggested in the Scientific Report submitted to the European Food Safety Authority (EFSA) (17). During this study, sera samples from 94 animals (24 pregnant heifers and 70 lactating cows) were initially tested. After the first sampling, farmers were informed about the test results. During the time until the repeated sampling, 21 of the pregnant heifers had given birth, thus becoming dairy cows, but three heifers were culled. In the second testing, the number of animals was smaller due to culling for various reasons. No predominant reason for culling was found. In total, 79 animals were tested during the follow-up.

Blood samples (3 mL) were collected by caudal venepuncture in sterile vacutainers containing a clot activator. Skin was cleaned before puncture by using 70% ethanol wipes. The serum samples were investigated for the presence of *C. burnetii* antibodies by a commercial ID Screen Q Fever Indirect Multi-species ELISA kit (ID.Vet, France; sensitivity of test: 100%, specificity of test: 100%). A serum sample was determined to be positive if S/P% > 50%, a sample was determined to be suspicious if 40% > S/P% ≤ 50%, and negative if S/P% ≤ 40%. *C. burnetii* phase specific antibodies were investigated by using a commercial VetLine Coxiella Phase I ELISA kit and VetLine Coxiella Phase II ELISA kit for research use only (Novatec, Germany; the declared sensitivity of both tests: 96.20%, specificity of both tests: 94.47%).

Milk samples (approx. 20 mL) were collected from all udder quarters in sterile plastic containers. The milk samples were collected from the same animals as for the blood. The tip of the teat was cleaned before sampling by using 70% ethanol wipes. The first milk jet was rejected before sampling. DNA from the milk samples for real time PCR was extracted manually with a QIAamp DNA Mini kit (Qiagen, Germany) using 100 μL elution volume. The Adiavet Cox Real time qPCR kit (Bio-X Diagnostics, France) was employed according to the provided protocol to amplify the IS1111 DNA sequence and GAPDH, as an internal control of extraction and amplification steps. A Rotor-Gene Q real-time PCR cycler (Qiagen, Germany) was used. During this study, milk samples from 69 animals were initially tested, and from 58 animals during the follow-up.

Table 1

Sampling and testing scheme

Target animals	Nature of sample	Type of analysis	Number of animals in each farm at first sampling
Cows having aborted recently or having stillborn calves and cows presenting reproduction problems: late returns, repeated artificial insemination etc.	Blood and milk	Serology by ELISA for blood samples; PCR for milk samples	2-4
Control cows: those showing no reproductive problems			6-8

According to the test results for sera and milk, all herds were divided into three categories: A: both seropositive and shedders (at least one animal), B: seropositive, no shedders, C: seronegative, no shedders.

Statistical analysis

The questionnaire results about farming practices and additional data regarding variables such as the age of the farm, the number of animals in the herd, the population density of ruminants in the local region (data obtained from the Agricultural Data Centre (18)), the frequency of bedding material (litter) change in the parturition area, and the time of placenta removal were analysed using the F-Test and the T-Test (MS Excel) in order to detect any significant differences of these variables between negative and positive farms. In the case of other factors, the chi-squared test and odds ratio calculators were used, as well as the 95% confidence interval for the apparent prevalence of disease was calculated according to the Wilson's algorithm (19) using the Epitools open access program (20). The relation between serological response and shedding of *C. burnetii* DNA in milk was evaluated by means of the odds ratio. For all statistically significant variables from the univariable analysis, the Principal Component Analysis and heatmap analysis using correlation distance and average linkage was performed by using ClustVis 2.0 software suite (21). Additionally, binary logistic regression analysis using Generalised linear models with binomial family and logit link was performed for all statistically significant variables identified from the univariable analysis by using the R base package (22). Besides the statistically significant variables, variables with a p-value below 0.20 in the univariable analyses were also included in these models, as described by Schimmer et al. (23).

Results

The data obtained from the questionnaire showed that Q fever positive and negative herds had significant differences in terms of reproductive problems. The average percentage of dystocia and stillborn calves, and the average percentage of abortions during the last year were significantly higher in positive herds than in the negative herds (Table 2). The percentage of dystocia in negative farms ranged from zero to 3.75%, but in positive farms – from zero to 30.00%. The percentage of stillborn calves in the negative farms ranged from zero to 6.00%, but in positive farms - from zero to 24.00%. Nine (64.28%) of the negative herds did not report abortions during the last year, while the rest of the herds reported that 1.00-6.00% of cattle suffered from abortions during the last year. In turn, four (16.00%) of the Q fever positive farms did not report abortions. The rest of the positive farms reported that 0.50-20.00% of cattle suffered from abortions during the previous year.

The number of animals in the herd, the regional population density of ruminants, artificial ventilation system in the farm, hoof trimming, and frequent farm visitors were found to be significant ($p<0.05$) factors for the presence of Q fever infection (Table 3). Deratisation was significantly less practised in negative farms, and the presence of ticks on dairy cattle was more often observed in negative farms. Frequent farm visitors means that farms frequently or occasionally allow visitors for seminars and excursions, as opposed to situations where farms do not allow visitors at all. Ticks were observed more often in negative farms, probably due to the fact that grazing in these farms was practised in 92.86% of cases (13 herds) *vs*. in 60.00% (15 herds) of positive farms. In total, 76.00% (19 herds) of positive farms performed deratisation, as opposed to only 35.71% (5 herds) of negative farms. In two negative farms, the only deratisation agent employed were cats (as it was considered by the farmer). In turn, the positive farms used rat poison, or deratisation was outsourced to specialised services.

The rest of the factors analysed also were not found to significantly affect the occurrence of infection in the herd (Table 3). The procedures for the removal of aborted foetus and placenta were evaluated as optimal *vs*. not optimal. The procedures were considered optimal if the aborted foetus and placenta were either sent for laboratory analysis in cooperation with a

Table 2

Data from the questionnaire about selected reproductive problems within the past year

Reproductive problem	Average in positive herds (n=25)*	Average in negative herds (n=14)**	p-value (t-test)
Cows with observed dystocia, %	5.98	1.17	**0.001**
Stillborn calves from all parturitions, %	4.81	2.11	**0.01**
Abortions, %	3.56	1.00	**0.005**

**Total number of cattle 14 570*

***Total number of cattle 975*

veterinarian, or placed in a designated container and later collected by a specialised disposal company, or incinerated or buried. The procedure was considered not to be optimal if the aborted foetus and placenta was fed to dogs or thrown into manure pits where they were accessible to domestic or wild animals.

The results of Principal Component Analysis obtained using ClustVis 2.0 for all significant risk

Table 3

Results of the questionnaire about farming practices in positive (n=25) and negative (n=14) herds

Risk factor	Positive herds	Negative herds	p-value*	Odds Ratio (95% CI)
Average age of the farm, years	14.56	17.50	0.15	-
Average number of animals in the herd, n	607.08	69.54	**0.00003**	-
Population density of ruminants in the local region, animals/km^2	10.26	6.91	**0.0007**	-
Imported animals in the herd, %	50.00	42.86	0.93	1.33 (0.35-5.03)
Origin of imported animals - Latvia vs. other countries, %	50.00 (n=12)	83.33 (n=6)	0.39	5 (0.44-56.62)
Vaccination and/or quarantine of imported animals, %	50.00 (n=12)	33.33 (n=6)	0.87	0.5 (0.07-3.85)
Number of herds with tie-stall/free stall/mixed	22.73 / 59.09 / 18.18 (n=22)	42.86 / 42.86/ 14.29 (n=14)	0.44	-
Number of open barn/closed barn	40.00 / 60.00	50.00 / 50.00	0.79	0.67 (0.18-2.49)
No designated parturition zone, %	36.36	71.43	0.09	0.23 (0.05-0.97)
No separate space for animals that suffered from abortion or are sick, %	54.55	78.57	0.27	0.33 (0.07-1.51)
Artificial ventilation system, %	40.00	0	**0.02**	19.65 (1.05-366.44)
Bedding material change in the parturition area, times per year	488.62	495.31	0.47	-
Do not use disinfectants in the parturition area, %	28.00	64.29	0.06	0.22 (0.05-0.88)
Placenta removal time, h	5.30	11.43	0.07	-
Hygienic measures at delivery - hand washing, %	88.00	100.00	0.47	4.51 (0.22-93.91)
Hygienic measures at delivery - use of rubber gloves, %	80.00	78.57	1.0	0.92 (0.18-4.58)
Hygienic measures at delivery - external genital washing, %	80.00	57.14	0.25	0.33 (0.08-1.41)
Hygienic measures at delivery - site preparation, %	68.00	78.57	0.74	1.73 (0.37-7.96)
Not optimal procedure with aborted foetus and placenta, %	17.39	28.57	0.7	0.53 (0.11-2.56)
Deratisation not performed, %	24.00	64.29	**0.03**	0.18 (0.04-0.73)
Hoof trimming, %	96.00	57.14	**0.01**	18 (1.87-173.04)
Hoof trimming outsourced, %	79.16 (n=25)	87.50 (n=8)	1.0	0.54 (0.05-5.5)
Sharing agricultural equipment with other farms, %	16.00	28.57	0.6	0.48 (0.1-2.31)

Risk factor	Positive herds	Negative herds	p-value*	Odds Ratio (95% CI)
Use of manure to fertilise agricultural lands, %	92.00	92.86	1.0	0.88 (0.07-10.72)
Grazing practised, %	60.00	92.86	0.07	8.65 (0.97-77.11)
Ticks observed on dairy cattle, %	20.00	78.57	**0.001**	0.07 (0.01-0.34)
Artificial insemination outsourced, %	40.00	57.14	0.56	0.54 (0.14-2.03)
Veterinarian outsourced (in opposite to permanently employed veterinarian), %	36.00	57.14	0.35	0.42 (0.11-1.61)
Permanent veterinarian but working in other farms as well, %	60.00	92.86	0.07	0.12 (0.01-1.03)
Farm visitors allowed, %	68.00	28.57	**0.04**	5.31 (1.27-22.24)

**Yates corrected Chi-square for two variables or t-test.*

factors are given in Figure 1, while the variance data explained by principal components and component loadings are given in Table 4. In PC1, the factor with the largest variance was the presence of an artificial ventilation system. In PC2, the factor with the largest variance explained was the average number of animals in the herd. The results of the heatmap analysis are given in Figure 2. It can be seen that seven statistically significant factors included in the analysis gave a good grouping of negative and positive farms. According to the results from the Generalised linear model, only two variables showed significant correlations with the infection risk on the farms – a positive correlation with the farm size (p = 0.03, coefficient 0.04) and a negative correlation with ticks observed on dairy cattle (p = 0.04, coefficient -3.50, AIC = 20.53).

The serological response on 10 selected positive farms identified with the ID.Vet kit and shedding *C. burnetii* DNA in milk showed an increase over six months, but this increase was statistically insignificant (Table 5). Table 6 provides an overall characterisation of 10 selected herds, with a focus on reported reproductive problems. Evidently, abortions were rare but stillborn calves and the need for repeated artificial insemination

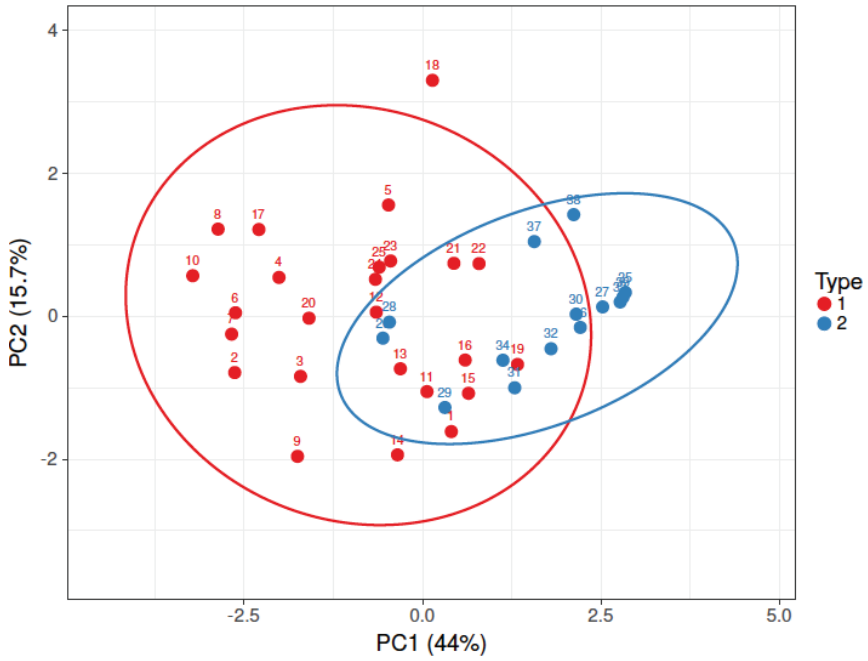


Figure 1. The results of Principal Component Analysis obtained using ClustVis 2.0 for all significant risk factors. Type 1 – positive farms. Type 2 – negative farms. Prediction ellipses indicate a probability of 0.95 that a new observation from the same group will fall inside the ellipse. N = 39 data points.

Table 4

Variance explained by principal components and component loadings for all significant risk factors

Dimensions	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Individual	0.44	0.16	0.11	0.10	0.08	0.05	0.05
Cumulative	0.44	0.60	0.71	0.82	0.90	0.95	1.00
Average number of animals in the herd	0.32	-0.57	0.22	-0.02	0.56	-0.43	0.15
Population density of ruminants in the local region	0.34	0.36	0.70	-0.08	-0.07	-0.13	-0.48
Artificial ventilation system	0.43	-0.07	0.15	-0.47	-0.46	0.14	0.58
Deratisation not performed	0.36	0.15	-0.57	-0.56	0.21	-0.07	-0.39
Hoof trimming	0.40	0.29	-0.31	0.51	-0.27	-0.56	0.16
Ticks observed on dairy cattle	-0.42	-0.30	0.02	-0.32	-0.47	-0.59	-0.22
Farm visitors	0.35	-0.58	-0.11	0.30	-0.37	0.33	-0.43

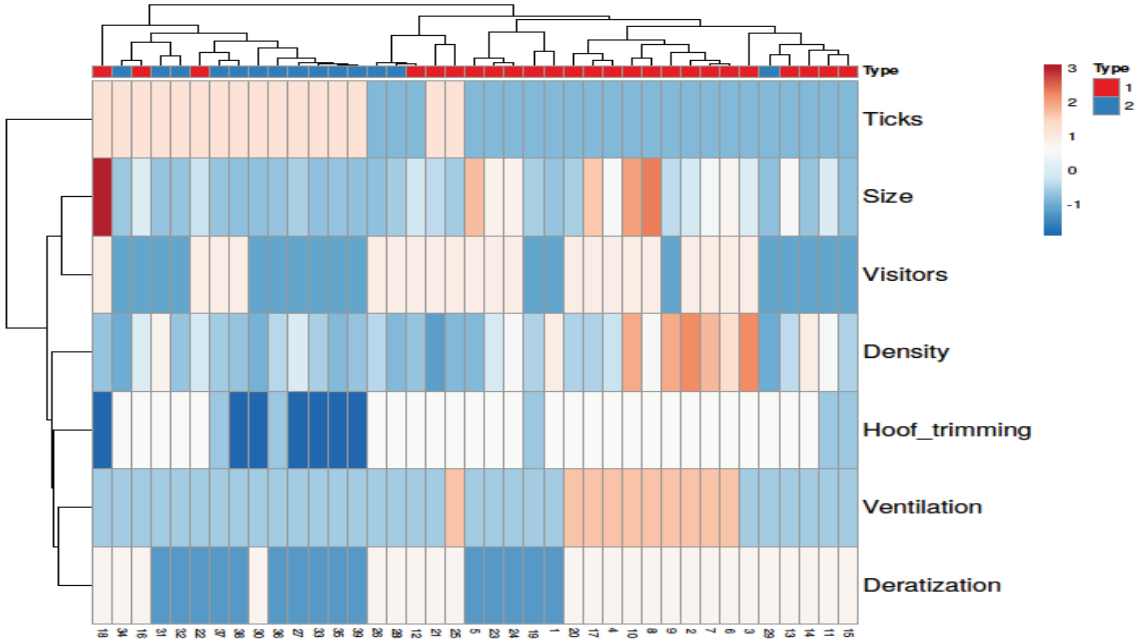


Figure 2. The heatmap analysis using correlation distance and average linkage using ClustVis 2.0 for all significant risk factors in all farms that filled the questionnaire. Explanations: Type 1 – positive farms; Type 2 – negative farms; Ticks – ticks observed on dairy cattle; Size – number of animals in the herd; Visitors – farm visitors; Density – Population density of ruminants in the local region; Hoof trimming – hoof trimming performed; Ventilation – artificial ventilation system operating; Deratisation – deratisation performed; farm numbering is the same as in Figure 1; intensity of colour gives an overview of the numeric differences.

were more common for the particular animals included in the study. In total, 32 animals (34.04%) out of 94 showed at least one positive test result during the whole study period (Table 6). The phase-specific serological $PhI^{-}/PhII^{+}$ pattern indicating an acute infection, but giving a negative result with the ID.Vet kit, was only observed in one cow from herd No. 10, but the animal had been culled by the time of the second sampling (Table 7). The $PhI^{+}/PhII^{+}$ pattern (including suspicious samples) indicating a chronic infection, and being positive according to the ID.Vet test, was observed in two cows from herd No. 6. At the time of the second sampling they were $PhI^{-}/PhII^{-}$ but still positive with the ID.Vet test. None of these three animals were shedding *C. burnetii* DNA in milk at the first sampling. At the second sampling, one of the animals was still not shedding the bacteria, but another one was a dry cow. The $PhI^{+}/PhII^{-}$ pattern (including suspicious samples)

Table 5

Dynamics of serological response and shedding of *C. burnetii* in milk tested initially (blood samples n = 94, milk samples n = 69) and the second time (blood samples n = 79, milk samples n = 58)

Evaluated parameter	Number of positive and suspicious animals initially (%, 95% CI)	Number of positive and suspicious animals at second sampling* (%, 95 % CI)
Antibodies (ID.Vet)	19 (20.21%, 13.34-29.43)	25 (31.65%, 22.45-42.55)
PhI^{+} (*NovaTec* Phase I)	6 (6.38%, 2.96-13.23)	5 (6.33%, 2.73-13.97)
PhII^{+} (*NovaTec* Phase II)	3 (3.19%, 1.09-8.97)	2 (2.53%, 0.7-8.77)
C. burnetii DNA in milk	3 (4.35%, 1.49-12.02)	6 (10.34%, 4.83-20.79)

**The samples were taken two times with an interval of 7.5 to 13 months.*

and all ID.Vet positive samples were identified in four cases from herds No. 3, 6, and 7. Two of the animals were shedding bacterial DNA at both sampling times, but the third one was not shedding at all. The seropositive heifer from herd No. 3 at the first sampling was culled by the time of the second sampling. Among 24 pregnant heifers at the first sampling, seven were negative and later become positive when tested the second time.

The herds were divided into three categories by the testing results of sera and milk: A: both seropositive and shedders (at least one animal), B: seropositive, not shedders, C: seronegative, not shedders. During this study, the number of herds in category A increased from 3 to 5, B decreased from 4 to 2, and C remained unchanged (3 and 3, respectively) (Table 6).

Table 6

The number of tested animals with previously recorded reproductive problems in each herd, the number of animals seropositive for Q fever and shedding *C. burnetii* DNA in milk at both sampling times, the number of animals that seroconverted, and herd category at the initial and repeated testing

Herd	Number of animals	Tested animals with reproductive problems, n			Seropositive animals initially, n	Shedding animals initially, n	Herd category initially	Seropositive animals repeatedly, n	Shedding animals repeatedly, n	Seroconverted animals, n	Herd category repeatedly
		Abortions	Stillborn calves	Repeated artificial insemination							
1	10	1	0	1	0	0	C	0	0	0	C
2	10	1	1	3	4	0	B	4	1	2	A
3	10	0	2	0	1	0	B	0	0	0	C
4	10	0	4	4	6	1	A	8	1	1	A
5	7	0	3	0	3	0	B	2	0	0	B
6	10	0	5	0	3	1	A	6	1	3	A
7	7	0	4	1	2	1	A	2	2	0	A
8	10	0	2	4	0	0	C	1	0	1	B
9	10	0	1	5	0	0	C	2	1	2	A
10	10	0	4	5	1	0	B	0	0	0	C
Total	94	2	26	23	19	3	-	25	6	9	-

Table 7

Detailed information about animals that were seropositive and/or shedding *C. burnetii* DNA in milk in every herd (all animals in herd No. 1 were negative)

Herd	Animal	First sampling				Second sampling		
		PhI/PhII	ID.Vet	*C. burnetii* DNA in milk	Reproductive problems	PhI/PhII	ID.Vet	*C. burnetii* DNA in milk
2	1	$PhI^{-}/PhII^{-}$	+	-	None	$PhI^{-}/PhII^{-}$	+	dry period
	2	$PhI^{-}/PhII^{-}$	+	-	Abortion	culled		
	3	$PhI^{-}/PhII^{-}$	+	-	Stillborn calf	$PhI^{-}/PhII^{-}$	+	-
	4	$PhI^{-}/PhII^{-}$	-	-	None	$PhI^{-}/PhII^{-}$	+	dry period
	5	$PhI^{-}/PhII^{-}$	+	-	None	culled		
	6	$PhI^{-}/PhII^{-}$	-	heifer	repeated AI**	$PhI^{-}/PhII^{-}$	+	+
3	1	$PhI^{susp}/PhII^{-}$	+	heifer	None	culled		
4	1	$PhI^{-}/PhII^{-}$	susp.*	-	None	$PhI^{-}/PhII^{-}$	susp.	dry period
	2	$PhI^{-}/PhII^{-}$	-	-	Stillborn calves in 3rd, 4th lactation	$PhI^{-}/PhII^{-}$	susp.	-
	3	$PhI^{-}/PhII^{-}$	+	+	Stillborn calf in 1st lactation	$PhI^{-}/PhII^{-}$	+	-
	4	$PhI^{-}/PhII^{-}$	+	-	None	$PhI^{-}/PhII^{-}$	+	-
	5	$PhI^{-}/PhII^{-}$	+	-	repeated AI	$PhI^{-}/PhII^{-}$	+	+
	6	$PhI^{-}/PhII^{-}$	+	-	Stillborn calf in 1st lactation	$PhI^{-}/PhII^{-}$	+	-
	7	$PhI^{-}/PhII^{-}$	-	heifer	repeated AI	$PhI^{-}/PhII^{-}$	+	-
	8	$PhI^{-}/PhII^{-}$	+	-	Stillborn calves in 4th, 5th lactation	$PhI^{-}/PhII^{-}$	+	-
5	1	$PhI^{-}/PhII^{-}$	+	-	Stillborn calf in 2nd lactation	$PhI^{susp}/PhII^{+}$	+	dry period
	2	$PhI^{-}/PhII^{-}$	+	-	None	$PhI^{-}/PhII^{-}$	-	dry period
	3	$PhI^{-}/PhII^{-}$	+	-	Stillborn calf in 2nd lactation	$PhI^{-}/PhII^{-}$	+	dry period
6	1	$PhI^{-}/PhII^{-}$	-	heifer	None	$PhI^{-}/PhII^{-}$	+	-
	2	$PhI^{+}/PhII^{-}$	+	-	None	$PhI^{susp}/PhII^{-}$	+	-
	3	$PhI^{-}/PhII^{-}$	-	+	Stillborn calf in 1st lactation	$PhI^{-}/PhII^{-}$	-	-
	4	$PhI^{-}/PhII^{-}$	-	heifer	None	$PhI^{-}/PhII^{-}$	+	dry period
	5	$PhI^{susp}/PhII^{+}$	+	-	Stillborn calf in 1st lactation	$PhI^{-}/PhII^{-}$	+	-
	6	$PhI^{-}/PhII^{-}$	-	heifer	None	$PhI^{-}/PhII^{-}$	+	+
	7	$PhI^{susp}/PhII^{susp}$	+	-	Stillborn calf in 1st lactation	$PhI^{-}/PhII^{-}$	+	dry period

Herd	Animal	First sampling				Second sampling		
		PhI/PhII	ID.Vet	*C. burnetii* DNA in milk	Reproductive problems	PhI/PhII	ID.Vet	*C. burnetii* DNA in milk
7	1	$PhI^{+}/PhII^{-}$	+	-	Stillborn calf in 4th lactation	$PhI^{-}/PhII^{-}$	+	-
	2	$PhI^{+}/PhII^{-}$	+	+	All calves died, stillborn calf in 3rd lactation	$PhI^{susp}/PhII^{-}$	+	+
	3	$PhI^{-}/PhII^{-}$	-	-	Stillborn calves in 2nd and 3rd lactation	$PhI^{-}/PhII^{-}$	-	+
8	1	$PhI^{-}/PhII^{-}$	-	heifer	repeated AI	$PhI^{susp}/PhII^{-}$	+	-
9	1	$PhI^{-}/PhII^{-}$	-	-	Stillborn calf in 2nd lactation	$PhI^{-}/PhII^{-}$	+	-
	2	$PhI^{-}/PhII^{-}$	-	heifer	None	$PhI^{susp}/PhII^{+}$	+	+
10	1	$PhI^{-}/PhII^{+}$	-	-	repeated AI	culled		

***** *susp. – suspicious*

*** – AI, artificial insemination.*

Discussion

The rate of abortion is a warning sign for cattle herds regarding the possible Q fever infection if it reaches three abortions per year in herds with less than 100 animals and more than 4% of cows suffering from abortions in herds with more than 100 animals (17). Among the Q fever negative farms participating in the survey, this level was reached or exceeded in two herds (in 14.29% of all negative herds), both having less than 100 animals. This could be explained by the fact that many other causative agents of abortions in cattle have been reported in Latvia, for example, *Listeria monocytogenes* (24). Among Q fever positive farms, the above-mentioned level was reached in 11 herds (44%), 10 of them having more than 100 animals. These data illustrate that attention must be paid to a series of abortions because this can indicate the presence of *C. burnetii* as well as other pathogens in the herd.

The investigation of farming practices and other risk factors by using the questionnaire and statistical analysis showed that several factors significantly increased the risk of a herd to be Q fever positive – such as the number of animals in the herd, the regional population density of ruminants, the operation of an artificial ventilation system in the farm, and frequent farm visitors. A herd size larger than 100 or 200 animals has been recognised as an important risk factor in several previous studies from many locations, for example, Northern Ireland (25), the Republic of Ireland (26), Denmark (11), the Netherlands (13), Portugal (27), Latvia (16), and Italy (28). Larger herd size might sustain the circulation of *C. burnetii* for an extended period as more animals are exposed to it while susceptible. The regional population density of ruminants or cattle in particular has also been previously identified as a factor that promotes the spatial propagation of the disease, for example, in Sweden (14), France (29), and Denmark (30).

According to the questionnaires, an artificial ventilation system was present in 40.00% of Q fever positive farms but in none of the negative farms. A higher prevalence of Q fever among stables that use forced ventilation has been detected on goat farms in the Netherlands (23). In general, artificial ventilation systems are more commonly used on larger farms (31).

Hoof trimming was performed significantly more often in positive farms but the outsourcing of this procedure vs. performing by a permanent employee of the farm did not increase the possibility of the farm being Q fever positive, contrary to the findings from a study caried out in Denmark (11). Nevertheless, hoof trimming equipment must be rigorously washed and disinfected after every farm visit.

Ticks were more often observed by farmers in negative farms, probably due to the fact that grazing was practised in these farms more widely. The presence of ticks has been correlated with Q fever in herds, for example in the Netherlands (13) and Cyprus (32). In both studies, the presence of cows with ticks or an unknown tick status was associated with the increased possibility of the bulk tank milk samples or abortion material being Q fever positive by PCR

while the presence of ticks generally correlated with grazing practices or seasonal changes. The presence of infected ticks has been indicated as a possible source for Q fever infection (33), and it has been experimentally proven that hard ticks such as *Ixodes ricinus* can transmit *C. burnetii* transstadially and might act as a vector in some instances (34). The role of ticks in the distribution of Q fever among Latvian dairy cattle farms should be additionally studied in future investigations.

Deratisation was statistically significantly more often not performed in negative farms. In two cases of negative farms, the only aid for deratisation was cats. The cats in fact are not a deratisation tool, but this is how it was considered by the farmers. In turn, the positive farms more often used rat poison, or deratisation was outsourced to specialised companies. Deratisation is an important hygiene measure and Q fever positive rats have been detected at many farms in the Netherlands (35). The impact of deratisation efforts on Q fever in Latvian farms should be studied more deeply in future investigations.

Farm visitors were allowed significantly more often in Q fever positive farms. A visitor ban has been identified as one of the long-term control options for Q fever, first of all to prevent human infections, but also as a part of the general biosecurity measures to limit the introduction and spread of various infectious diseases of animals in general (5).

In total, 32 (34.04%) out of 94 animals from 10 selected farms showed at least one positive test result during the whole study period. Among the serum samples collected at 1st sampling (n=94), serological evidence of *C. burnetii* infection was detected in 19 (20.21%) cases by ID.Vet. *C. burnetii* Phase I and Phase II specific antibodies were observed in 6 (6.38%) and 3 (3.19%) of the investigated samples, respectively. The larger number of positive animals revealed by ID.Vet can be explained by the fact that the ID.Vet kit has a higher sensitivity and specificity, thus it is suitable in the screening samples for *C. burnetii* antibodies but it does not discriminate Phase I and Phase II antibodies. The sensitivity declared by the producer of the ID.Vet kit is 100%, while the sensitivity of NOVATEC ELISA is only 96.20%.

In the first sampling, only one of the seropositive animals was a pregnant heifer; the rest were lactating cows. This finding is in a good agreement with another study where the highest seroprevalence was detected in the age group of 6-8 years (4).

Among the 79 sera samples collected at the 2nd sampling, serological evidence of *C. burnetii* infection was detected in 5 (6.00%) and 2 (3.00%) cases by the "*NovaTec* Phase I" and "*NovaTec* Phase II" respectively, while ID.vet revealed 25 (32.00%) positive samples. However, the increase in the numbers of seropositive animals tested by ID.vet was not statistically significant. This is in line with another study (36) where antibody levels in dairy cattle were rather stable over 11 months. In spite of this, increased individual seroprevalence over time has also been described (34). In our study, four of the initially seropositive animals had been culled before the time of the second sampling. Two of the initially seropositive animals had converted to seronegative when tested the second time. Fourteen of the initially seropositive animals were seropositive at the second testing as well. During the second sampling, nine new cases were detected, with seven of them being primiparous cows (pregnant heifers during the first sampling, respectively). These new cases can be explained by the typical route of infection – inhalation of *C. burnetii* shed by infected animals during delivery. The slightly increased seroprevalence in heifers has been reported in other studies as well (37).

The results showed shedding of *C. burnetii* DNA in milk in 3 (4.00%) cows when tested initially. Two of the shedders were seropositive with ID.vet, one of these two was seronegative according to the "*NovaTec* Phase" kits but the second was $PhI^{-}/PhII^{+}$. One of the shedders was seronegative with all serological tests. The relatively low number of shedders compared to the number of seropositive animals has been described by other authors as well as shedding of *C. burnetii* by seronegative animals as well (38).

Results when tested repeatedly showed shedding of *C. burnetii* DNA in the milk of 6 (10%) cows, increasing from three at the first sampling. This increase was statistically not significant. This is in line with other studies (34) where the prevalence levels of *C. burnetii* organisms were rather stable. Five of the shedders were seropositive as well, besides two of them were primiparous cows. Two of the shedders initially became non-shedders during 7.5 to 13 months. This is understandable because two of predominant shedding patterns – persistent and sporadic – have been described (8).

The $PhI^{+}/PhII^{-}$ pattern (including suspicious samples) and all ID.Vet positive samples were detected in four cases from herds No. 3, 6, and 7. Two of the animals were shedding *C. burnetii* DNA at both sampling times, but the third one was not shedding at all. The animal from herd No. 3 was not in milk at the first sampling and was culled prior to the second sampling. The $PhI^{+}/PhII^{-}$ pattern was rarely detected in a study from Germany (4). A conclusion was made that it emerged from the $PhI^{+}/PhII^{+}$ pattern, with the largest number of these animals later reverting to negative, apparently by building up cellular immunity (4). In our investigation, these animals were still

positive as detected by the ID.Vet kit at the second sampling as well.

The probability of initially seropositive or suspicious cows also being shedders of *C. burnetii* in milk was 33 times higher than the seronegative ones using "*NovaTec* Phase I" and 7 times higher using ID.vet (OR 32.5 for "*NovaTec* Phase I" and 7.43 for ID.vet). The latter probability is quite similar to the findings from another study where OR was around 9 (38). When tested the second time, the probability of seropositive cows also being shedders was 19 times higher than the seronegative ones using ID.vet (OR 18.63).

When analysing the changes in the herd status (A, B, C), the conversion from B to C (2 herds) can be attributed to the culling of initially seropositive animals. The conversion from B or C to A can be explained by initially seronegative heifers becoming both seropositive and shedding primiparous cows. The increasing prevalence of seropositive dairy herds over time has been reported in other studies as well (30). It must be admitted that not only *C. burnetii* may cause reproduction problems. In general, farmers in Latvia have the possibility and even an obligation to send every aborted foetus and a blood sample of the aborted cow for the tests of brucellosis, listeriosis, leptospirosis, Q fever, campylobacteriosis, and Schmallenberg virus. In the case of 94 animals included in the present study, since the farmers wanted to protect the confidentiality of their property, the exact data for all historically aborted cattle was not available.

Conclusions

Only two of the analysed risk factors that were significant for Q fever infection in dairy herds can be readily mitigated – the ventilation system and frequent farm visitors. Other significant risk factors such as the herd size and regional population density of ruminants cannot be radically altered, but they have to be taken into account by farmers, veterinarians, and decision makers. Proactive measures performed to limit the infection, such as the culling of seropositive animals or shedders can give positive results, eliminating new cases of infection over time. By analysing the dynamics of infection within herds, it was concluded that without any disease-limiting measures the number of both seropositive and shedding animals increases. The young animals (heifers) are most probably infected during delivery when they are exposed to bacteria that have been spread in the environment by older infected animals.

References

Agger, J.F., Paul, S. (2014). Increasing prevalence of *Coxiella burnetii* seropositive Danish dairy cattle herds. *Acta Vet Scand*. 56(1): 1–4. DOI: 10.1186/s13028-014-0046-2

Agricultural Data Centre: Statistics 2020. Retrieved March 3, 2021, from http://pub.ldc.gov.lv

Alvarez, J., Perez, A., Mardones, F.O., Pérez-Sancho, M., García-Seco, T., Pagés, E., … Domínguez, L. (2012). Epidemiological factors associated with the exposure of cattle to *Coxiella burnetii* in the Madrid region of Spain. *Vet J.* 194(1): 102–107. DOI: 10.1016/j.tvjl.2012.02.022

Anastácio, 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 Emerging Dis*. 63(2): e293-e300. DOI: 10.1111/tbed.12275

Angelakis, E., Raoult, D. (2010). Q fever. *Vet Microbiol.* 140(3-4): 297–309. DOI: 10.1016/j.vetmic.2009.07.016

Angen, Ø., Ståhl, 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(12): 5750–5759. DOI: 10.3168/jds.2011-4197

Barberio, A., Badan, M., Busa, A., Ceglie, L., Capello, K., Comin, A., … Natale, A. (2014). Association between serological response and shedding of *Coxiella burnetii* in milk in dairy cattle. *Large Anim Rev.* 20(1): 3–8.

Barlozzari, G., Sala, M., Iacoponi, F., Volpi, C., Polinori, N., Rombolà, P., … Scarpulla, M. (2020). Cross-sectional serosurvey of *Coxiella burnetii* in healthy cattle and sheep from extensive grazing system in central Italy. *Epidemiol Infect.* 148:e9, 1-8. DOI: 10.1017/S0950268819002115

Boroduske, A., Trofimova, J., Kibilds, J., Papule, U., Sergejeva, M., Rodze, I., Grantina-Ievina, L. (2017). *Coxiella burnetii* (Q fever) infection in dairy cattle and associated risk factors in Latvia. *Epidemiol Infect.* 145(10): 2011–2019. DOI: 10.1017/S0950268817000838

Böttcher, J., Vossen, A., Janowetz, B., Alex, M., Gangl, A., Randt, A., Meier, N. (2011). Insights into the dynamics of endemic Coxiella burnetii infection in cattle by application of phase-specific ELISAs in an infected dairy herd. *Vet Microbiol.* 151(3–4): 291–300. DOI: 10.1016/j.vetmic.2011.03.007

Brown, L.D., Cat, T.T., DasGupta, A. (2001). Interval estimation for a binominal proportion. *Stat Sci.* 16: 101–33. DOI: 10.1214/ss/1009213286

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(1): 1–7. DOI: 10.1186/1746-6148-7-13

Capuano, F., Landolfi, M.C., Monetti, D.M. (2001). Influence of three types of farm management on the seroprevalence of Q fever as assessed by an indirect immunofluorescence assay. *Vet Rec.* 149(22): 669– 671. DOI: 10.1136/vr.149.22.669

EFSA Panel on Animal Health and Welfare (AHAW). (2010). Scientific Opinion on Q Fever. *EFSA Journal.* 8(5): 1595. DOI: 10.2903/j.efsa.2010.1595

Eldin, C., Mélenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., … Raoult, D. (2017). From Q fever to *Coxiella burnetii* infection: a paradigm change. *Clin Microbiol Rev.* 30:115–190. DOI: 10.1128/CMR.00045-16

Grantiņa-Ieviņa, L., Bubula, I., Sergejeva, M., Šteingolde, Ž., Boikmanis, G., Cīrule, D., … Bērziņš, A. (2021). Pilot study of risk group human seroprevalence to *Coxiella burnetii* (Q fever) in Latvia. *Proc. Latv. Acad. Sci. B: Nat. Exact Appl. Sci., Section B.* 75(5): 364–370. DOI: 10.2478/prolas-2021-0053

Guatteo, R., Beaudeau, F., Joly, A., Seegers, H. (2007). *Coxiella burnetii* shedding by dairy cows. *Vet Res.* 38(6): 849–860. DOI: 10.1051/vetres:2007038

Körner, S., Makert, G. R., Mertens-Scholz, K., Henning, K., Pfefer, M., Starke, A., … Ulbert, S. (2020). Uptake and fecal excretion of *Coxiella burnetii* by *Ixodes ricinus* and *Dermacentor marginatus* ticks. *Parasites Vectors.* 13(75): 2-11. DOI: 10.1186/s13071-020-3956-z

McCaughey, C., Murray, L.J., McKenna, J.P., Menzies, F.D., McCullough, S.J., O'Neill, H.J., … Coyle, P.V. (2010). *Coxiella burnetii* (Q fever) seroprevalence in cattle. *Epidemiol Infect*. 138(1): 21–27. DOI: 10.1017/S0950268809002854

Metsalu, T., Vilo, J. (2015). ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic Acids Res,* 43(W1): W566–W570. DOI: 10.1093/nar/gkv468

Mondaca, M.R. (2019). Ventilation systems for adult dairy cattle. *Vet Clin N Am-Food A.* 35(1): 139-156. DOI: 10.1016/j.cvfa.2018.10.006

Nusinovici, S., Frössling, 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(15): 3316–3326. DOI: 10.1017/S0950268814003926

Pandit, P., Hoch, T., Ezanno, P., Beaudeau, F., Vergu, E. (2016). Spread of *Coxiella burnetii* between dairy cattle herds in an enzootic region: modelling contributions of airborne transmission and trade. *Vet Res.* 47(1): 1–16. DOI: 10.1186/s13567-016-0330-4

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(1-2): 57–64. DOI: 10.1016/j.prevetmed.2012.05.015

Piñero, A., Ruiz-Fons, F., Hurtado, A., Barandika, J.F., Atxaerandio, R., García-Pérez, A.L. (2014). Changes in the dynamics of *Coxiella burnetii* infection in dairy cattle: An approach to match field data with the epidemiological cycle of *C. burnetii* in endemic herds. *J Dairy Sci.* 97(5): 2718–2730. DOI: 10.3168/jds.2013-7229

Plummer, P.J., McClure J.T., Menzies, P., Morley, P.S., Van den Brom, R., Van Metre, D.C. (2018). Management of Coxiella burnetii infection in livestock populations and the associated zoonotic risk: A consensus statement. *J Vet Intern Med.* 32(5): 1481-1494. DOI: 10.1111/jvim.15229

R Development Core Team. (2009). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Reusken, C., van der Plaats, R., Opsteegh, M., de Bruin, A., Swart, A. (2011). *Coxiella burnetii* (Q fever) in Rattus norvegicus and Rattus rattus at livestock farms and urban locations in the Netherlands; could Rattus spp. represent reservoirs for (re) introduction? *Prev Vet Med.* 101(1-2): 124-130. DOI: 10.1016/j.prevetmed.2011.05.003

Rodolakis A. (2009). Q Fever in Dairy Animals. *Ann NY Acad Sci.* 1166(1): 90–93. DOI: 10.1111/j.1749-6632.2009.04511.x

Ryan, E.D., Kirby, M., Collins, D.M., Sayers, R., Mee, J.F., Clegg, T. (2011). Prevalence of *Coxiella burnetii* (Q fever) antibodies in bovine serum and bulk-milk samples. *Epidemiol Infect.* 139 (9): 1413–1417. DOI: 10.1017/S0950268810002530

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(1): 1-14. DOI: 10.1186/1746-6148-7-81

Sergeant, ESG: Epitools Epidemiological Calculators. Ausvet. [software]. 2018 Retrieved May 21, 2021, from http://epitools.ausvet.com.au.

Sidi-Boumedine, K., Rousset, E., Henning, K., Ziller, M., Niemczuck, K., Roest, H.I.J., Thiéry, R. (2010). Development of harmonised schemes for the monitoring and reporting of Q-fever in animals in the European Union. *EFSA Supporting Publications*. 7(5): 48E. DOI: 10.2903/sp.efsa.2010.EN-48

Steingolde, Z., Avsejenko, J., Berzins, A. (2014). Overview of *Listeria monocytogenes* caused abortions in cattle in Latvia in 2013. *Res for Rural Dev.* 1: 190-193. ISSN: 16914031

Taurel, A.F., Guatteo, R., Joly, A., Seegers, H., Beaudeau, F. (2011). Seroprevalence of Q fever in naturally infected dairy cattle herds. *Prev Vet Med.* 101(1-2): 51–57. DOI: 10.1016/j.prevetmed.2011.05.005

Van Engelen, E., Schotten, N., Schimmer, B., Hautvast, J.L.A., Van Schaik, G., Van Duijnhoven, Y.T.H.P. (2014). Prevalence and risk factors for *Coxiella burnetii* (Q fever) in Dutch dairy cattle herds based on bulk tank milk testing. *Prev Vet Med.* 117(1): 103-109. DOI: 10.1016/j.prevetmed.2014.08.016

World Organisation for Animal Health [Internet]. (2019). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019. Chapter 3.1.16. Q fever. C2019*. Retrieved May 21, 2021, from https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/

Supplementary material
The questionnaire used in the farm survey

No	Question
1	Age of the farm in years at the current size of the farm (change in the number of animals +/- 30% of the herd).
2	Have you bought cattle from other farms in Latvia and abroad?
3	The place from which the animals originated.
4	What measures were / are being taken for the imported animals on your farm? Several possible answers: no action/ vaccination/ quarantine.
5	Type of animal housing. Several possible answers: tie-stall/ free stall/ mixed.
6	Type of animal housing. Several possible answers: open barn/ closed barn.
7	Is there a separate parturition area?
8	Is there a separate holding area for sick animals (including animals that suffered from abortion)?
9	What kind of ventilation is in the shed?
10	How often is the bedding material changed in the delivery area (or in the holding place around the time of parturition)?
11	Are disinfectants used in the parturition area?
12	The time at which the placenta is removed from the parturition area.
13	Hygiene measures at delivery. Multiple choice possible: hand washing/ use of rubber gloves/ external genital washing/ delivery site preparation.
14	What is done with the foetus, placenta etc. in case of abortion?
15	How many cows out of 100 cows have had dystocia?
16	Percentage of stillbirths from all parturitions in the last year.
17	What percentage of dairy cows have had an abortion in the last year?
18	How often is deratization performed?
19	What deratization methods are used (for example, traps, poison)?
20	Hoof trimming. Several possible answers: regularly, irregularly, not performed.
21	Is hoof trimming outsourced?
22	Does the farm use only its own agricultural machinery (equipment)?
23	Do you use manure to improve the soil?
24	What groups of animals are on the pasture during the summer season? Multiple choice possible: dairy cows in dry period/ dairy cows/ young animals/ grazing is not practiced.
25	Have you noticed tick infestation in herd animals?

26 Organisation of artificial insemination. Several possible answers: only outsourced/ performed only by a permanent employee of the farm/ combined practices.

27 Veterinary services. Several possible answers: permanently employed veterinarian/ veterinarian outsourced.

28 Does the farm veterinarian also serve other farms?

29 Are there any events on the farm (seminars, tours, etc.)? Several possible answers: often/ rarely/ the farm does not accept visitors.

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