

Papers

Coxiella burnetii in bulk tank milk samples from dairy goat and dairy sheep farms in The Netherlands in 2008

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In 2007, a human Q fever epidemic started, mainly in the south eastern part of The Netherlands with a suspected indirect relation to dairy goats, and, to a lesser degree, to dairy sheep. This article describes the Q fever prevalences in Dutch dairy goat and dairy sheep bulk tank milk (BTM) samples, using a real-time (RT) PCR and ELISA. Results of BTM PCR and ELISA were compared with the serological status of individual animals, and correlations with a history of Q fever abortion were determined. When compared with ELISA results, the optimal cut-off value for the RT-PCR was 100 bacteria/ml. In 2008, there were 392 farms with more than 200 dairy goats, of which 292 submitted a BTM sample. Of these samples, 96 (32.9 per cent) were PCR positive and 87 (29.8 per cent) were ELISA positive. All farms with a history of Q fever abortion (n=17) were ELISA positive, 16 out of 17 were also PCR positive. BTM PCR or ELISA positive farms had significantly higher within-herd seroprevalences than BTM negative farms. In the south eastern provinces, the area where the human Q fever outbreak started in 2007, a significantly larger proportion of the BTM samples was PCR and ELISA positive compared to the rest of The Netherlands. None of the BTM samples from dairy sheep farms (n=16) were PCR positive but three of these farms were ELISA positive. The higher percentage of BTM positive farms in the area where the human Q fever outbreak started, supports the suspected relation between human cases and infected dairy goat farms.

Q fever is a zoonosis caused by *Coxiella burnetii*, which is an aerobic, obligate intracellular, Gram-negative, highly resistant bacterium that may infect mammals, birds, arthropods and man (Babudieri and Moscovici 1952, Arricau-Bouvery and others 2005, Berri and others 2007). In domestic ruminants, the primary animal reservoir of *C. burnetii*, the main clinical sign of Q fever is abortion. *C. burnetii* is mainly shed after parturition or abortion in birth products, but shedding also occurs in urine, faeces and milk (Arricau-Bouvery and others 2003, Guatteo and others 2007, García-Pérez and others 2009).

In 2007, a human Q fever epidemic started in the south eastern part of The Netherlands within three years resulting in almost 3500 officially notified human patients (van der Hoek and others 2010), and an indirect relation to dairy goats was suspected (Van Steenberg and

others 2007). Because of the precautionary principle, the Dutch government decided to implement measures on infected dairy sheep and goat farms, making it necessary to distinguish between infected and non-infected farms. In order to demonstrate an infection with *C. burnetii* in animals, individual tests like ELISA and real-time PCR (RT-PCR) in various matrices and immunohistochemistry (IHC) performed on placentas are available (Kováčová and Kazár 2000, Wouda and Dercksen 2007, García-Pérez and others 2009, Muskens and others 2011). Taking into account the size of the Dutch dairy goat farms with an average number of around 900 adult animals per farm (Van den Brom and Vellema 2009), a monitoring programme based on repeated individual testing is expensive and difficult to perform. However, for dairy cattle farms, bulk tank milk (BTM) sampling for different diseases, as neosporosis, salmonellosis and bovine viral diarrhoea (BVD), has been shown to be a good method to measure the disease status of lactating animals (Veling and others 2002, Zimmer and others 2002, Bartels and others 2005) and this has also been demonstrated for *C. burnetii* (Kim and others 2005, Muskens and others 2011). For cattle, it has been shown that shedding of *C. burnetii* occurs in milk, faeces and vaginal fluid. From these shedding routes, shedding by milk is the most continuous one (Guatteo and others 2007, 2011).

The aim of this study was (1) to determine the agreement between the results of a commercially available ELISA and RT-PCR in the same BTM samples and individual serum samples from dairy goat and dairy sheep farms with and without a history of IHC-confirmed Q fever abortions and (2) to describe the Q fever prevalence on farm level by testing BTM samples using this ELISA and RT-PCR, related to the results of individual blood samples and of IHC-confirmed Q fever abortions.

Veterinary Record (2012) 170, 310

doi: 10.1136/vr.100304

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Provenance: not commissioned;
externally peer reviewed.

Accepted January 1, 2012

Published Online First February 20, 2011



TABLE 1: PCR and ELISA results of dairy goat BTM samples

	Negative		Weak	PCR Positive	Very high	Total (95% CI)
	n	%				
ELISA	196		28	61	7	292
Negative	205	64.7%	4.5%	1.0%		70.2% (67.6 to 72.8)
Low positive	19	0.7%	3.1%	2.7%		6.5% (5.1 to 7.9)
Positive	61	1.4%	2.1%	15.4%	2.1%	20.9% (18.5 to 23.3)
High positive	7	0.3%		1.7%	0.3%	2.4% (1.5 to 3.3)
Total (95% CI)	292	67.1% (64.4 to 69.8)	9.6% (7.9 to 11.3)	20.9% (18.6 to 23.3)	2.4% (1.5 to 3.3)	100.0%

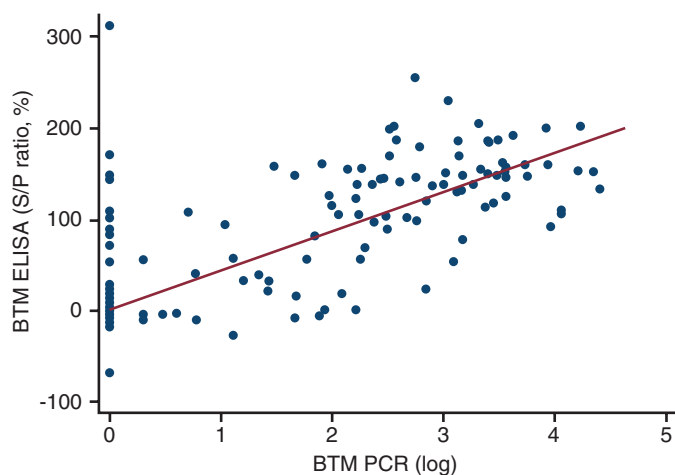


FIG 1: Comparison of semiquantitative PCR results (as log value) and ELISA results (S/P-ratio) of 292 goat and 16 sheep bulk tank milk samples. Note that the results of many samples are zero in both tests

Materials and methods

Study population in The Netherlands

In 2008, there were 40 professional dairy sheep farms and 392 dairy goat farms with more than 200 dairy goats per farm, containing approximately 260,000 goats in total (Van den Brom and Vellema 2009). No vaccination for *C burnetii* was performed on these farms before sampling.

Sampling

BTM samples

In 2008, all 392 dairy goat and 40 dairy sheep farmers in The Netherlands were asked to submit a BTM sample to be tested for *C burnetii*, using an ELISA and a RT-PCR.

Serum samples

Serum samples were submitted from randomly selected farms as part of the annual *Brucella melitensis* monitoring programme. Per farm, 13 samples from animals older than one year were tested. This number of animals is sufficient taking into account that during a Q fever abortion outbreak in goats, abortion rates up to 90 per cent are described (Palmer and others 1983, Hatchette and others 2003, Arricau-Bouvery and Rodolakis 2005, Van den Brom and Vellema 2009) and high seroprevalences are therefore expected on infected farms. The within-herd seroprevalences were determined on 77 of the farms that submitted a BTM sample. Correlations between the ELISA and PCR BTM results and the within-herd seroprevalences were investigated.

Farms with a history of abortion caused by *C burnetii*

In The Netherlands, abortion herd prevalences exceeding 5 per cent were notifiable in 2008. *C burnetii* was first diagnosed as abortifacient agent on a dairy goat farm in 2005. The diagnosis was made by IHC detection of *C burnetii* in sections of fetal membranes of representative cases. Since that time, suspected cases of Q fever abortions were tested by IHC. IHC was performed using the EnVision+ system (DAKO). For the first incubation step, sheep-anti-*C burnetii* IgG1, labelled with

horseradish peroxidase (HRP) was used, which was kindly provided by the Moredun Research Institute, Scotland, UK. The following step was incubation with rabbit anti-HRP and consecutively with the DAKO Envision+ system antirabbit. The immunoperoxidase staining was done with diaminobenzidine using the DAKO Liquid DAB+ Substrate-Chromogen System and sections

were counterstained with haematoxylin (Wouda and Dercksen 2007). On dairy goat farms where Q fever abortion was confirmed between 2005 and 2008, the relationship with the results of the BTM samples was investigated.

ELISA

In this study, BTM and serum samples were tested for the presence of antibodies to *C burnetii* with an indirect ELISA (Ruminants Serum Q Fever LSI Kit, LSI). The ELISA test is based on antigen obtained from an European ovine strain. The test was used according to the manufacturer's instructions. Briefly, serum was diluted 1:400, and milk was diluted 1:20 in dilution buffer, and both were transferred to 96 wells ELISA plates (total volume 100 µl), coated with antigen. The serum samples were incubated for one hour at 37°C and the milk samples overnight at 4°C. The plates were washed four times and incubated with 100 µl antiruminant IgG peroxidase conjugate for one hour at 37°C. After washing four times, the wells were incubated with 100 µl tetramethylbenzidine substrate for 10 minutes at 22°C in darkness. Colour development was stopped by the addition of 100 µl stop solution (0.5 M H₂SO₄). Optical density values were measured at 450 nm (OD₄₅₀). Sample/positive percentages (S/P per cent) were calculated using the following formula $(OD_{\text{sample}} - OD_{\text{negative control}}) / (OD_{\text{positive control}} - OD_{\text{negative control}}) \times 100$ per cent. The resulting S/P per cent for serum samples were divided in two different classes: negative (S/P per cent < 40) or positive (S/P per cent ≥ 40). For BTM samples, the different classes were as follows: negative (N; S/P per cent < 30), low positive (LP; 30 ≤ S/P per cent < 100), positive (P; 100 ≤ S/P per cent < 200) and high positive (HP; S/P per cent ≥ 200).

PCR

The BTM samples were tested using a commercial RT-PCR assay (LSI Taqvet *C burnetii*, Laboratoire Service International) which targets the repetitive transposon-like region of the bacterium. The test was used according to the manufacturer's instructions. DNase RNase free water was used as negative control sample. The external positive control sample was delivered with the kit and contained 10⁵ *C burnetii* ml (Strain CB01, INRA). DNA was extracted using the QIAmp DNA mini kit (Qiagen S.A) according to the manufacturer's instructions. The extraction was performed directly from 200 µl of raw milk. The PCR assays were performed using ABI Prism sequence Detection System 7500 (Applied Biosystems). For positive samples with a typical amplification curve, the results were given in Ct (cycle threshold) values. The samples presenting a typical amplification curve with a Ct value below 40 were considered to be positive. Each sample was also tested with a specific primer set for the ruminant household gene glyceraldehyde 3-phosphate dehydrogenase. Titres of *C burnetii*/ml were quantified. For each sample, quantification was based on a reference line generated in each test from decimal dilutions of the positive control. The results are presented in four classes: negative (N; no bacteria), weak positive (WP; 1 ≤ PCR < 100 bacteria/ml), high positive (HP; 100 ≤ PCR < 10,000 bacteria/ml), very high positive (VH; PCR ≥ 10,000 bacteria/ml).

Statistical data analysis

Farm prevalences for the presence of antibodies and the repetitive transposon-like regions of *C burnetii* were calculated. Corresponding 95 per cent CI were calculated with WinEpiScope 2.0 (Thrusfield and others 2001). Potential risk factors were analysed by logistic regression (logistic, STATA/SE 11.2). Bonferroni method was used for multiple

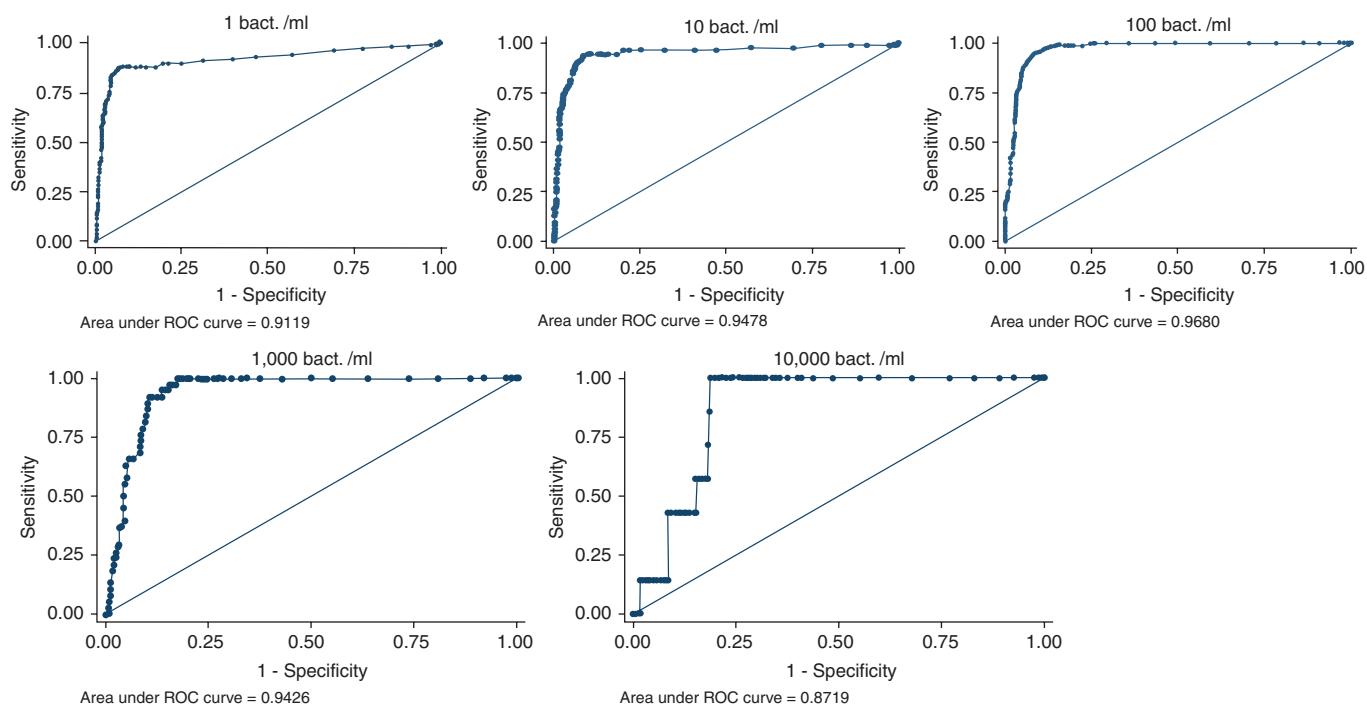


FIG 2: ROC plots of the BTM antibody ELISA with use of the BTM RT-PCR as reference value. Different plots indicate different cut-off levels of the BTM PCR of respectively 1, 10, 100, 1000 and 10,000 bacteria per ml

comparisons between ELISA or PCR classes and number of positive goats per farm (Oneway, Bonferroni, STATA/SE 11.2).

Results

Descriptive data for the BTM ELISA and RT-PCR

A total of 308 BTM samples from dairy sheep and dairy goat farms were tested by RT-PCR and ELISA. From the 292 goat BTM samples, 87 (29.8 per cent [95 per cent CI: 27.2 to 32.5]) were ELISA positive and 96 (32.9 per cent [95% CI: 30.2 to 35.6]) were PCR positive (Table 1). From the 16 sheep BTM samples, three (18.8 per cent [95 per cent CI: 4.0 to 33.6]) were ELISA positive and none were PCR positive. These BTM results were also used to determine the characteristics of the tests.

Results of BTM ELISA versus BTM PCR

Log-transformed quantitative PCR data were compared with ELISA S/P ratios and a correlation coefficient (r) of 0.90 was calculated (Fig 1). Different cut-off levels of the BTM PCR were taken as reference values. After this, for each PCR cut-off level Receiver Operator Curve (ROC) curves were plotted for the different BTM ELISA S/P ratios. For the chosen PCR cut-offs of 1, 10, 20, 50, 80, 100, 120, 200, 500, 1000, 2000, 5000 and 10,000, the highest area under the ROC curve of the ELISA was at a PCR cut-off of 100 bacteria/ml (Fig 2). For this reference value, the area under the ROC curve of the ELISA S/P ratio was 0.968 and the maximum proportion of agreement was reached at ELISA cut-off of 93 per cent S/P ratio reaching a sensitivity of 88.2 per cent and a specificity of 94.6 per cent. At cut-off levels of 30, 100 and 200 S/P ratios, as indicated by the manufacturer, the sensitivity and specificity was 95.6, 85.3 and 8.8 per cent, respectively, and 89.6, 95.0 and 99.6 per cent, respectively.

Individual serum samples

The overall percentage seropositive goats was 17.7 per cent. From the 77 herds, 40 (51.9 per cent [95 per cent CI: 41.9 to 61.9]) herds contained one or more positive animals out of 13 sampled animals. From these herds with positive samples, the mean prevalence was 4.4 and the median was four positive animals out of 13.

Correlation BTM ELISA and individual serum samples

Different cut-offs of within-herd seroprevalences were taken as reference for estimating the sensitivity and specificity of the BTM ELISA

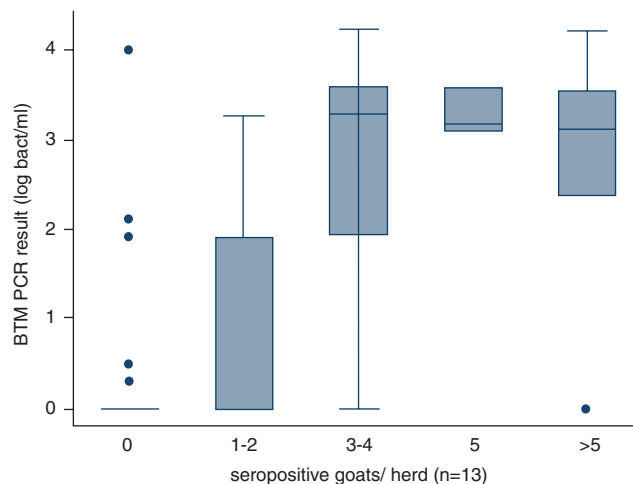


FIG 3: Comparison of the BTM PCR results (log value) and the number of seropositive animals, from 13 sampled animals, serologically tested by ELISA, per herd. Note that the results of many farms are 0 for both PCR and within-herd seroprevalence

with different S/P cut-off levels. For the chosen cut-off levels of prevalence (8, 15, 23, 46 and 62 per cent), the area under the ROC curve was highest (0.8774) for a within-herd seroprevalence of 15%. In that situation, the proportion of agreement was highest (88.3 per cent) at BTM ELISA cut-off of 46 per cent S/P ratio. At this cut-off, the sensitivity of the BTM ELISA was 84.3 per cent and the specificity was 91.1 per cent. The correlation coefficient between within-herd seroprevalences and BTM ELISA S/P ratio was $r=0.72$.

Correlation BTM PCR/ELISA and history of Q fever abortion

Results of the BTM PCR and ELISA in herds with IHC confirmed Q fever abortion were compared with results of herds without notified Q fever abortions. From 17 goat herds with a history of abortion, 16 (94.1 per cent [95 per cent CI: 83.2 to 100.0]) were BTM PCR positive and 17 (100.0 per cent) were BTM ELISA positive. In herds without notified Q fever abortion ($n=275$), 80 (29.1 per cent

TABLE 2: Serological results of individual goats, average number of positive animals per farm and herd prevalences in relation to BTM PCR results of these farms

BTM PCR	Number of farms	Average number of positive animals per farm	Herd prevalence	Number of serological positive goats per farm out of 13					
				0	1	2	3	4	≥5
Negative	45	0.80	6.2%	32	6	3	1	0	3
Weak	6	1.17	9.0%	3	1	1	0	1	0
Positive	24	5.13	39.4%	2	1	2	3	2	14
Very high	2	5.50	42.3%	0	0	0	0	1	1
Total	77	2.30	17.7%	37	8	6	4	4	18

BTM Bulk tank milk

TABLE 3: Serological results of individual goats, average number of positive animals per farm and herd prevalences in relation to BTM ELISA results of these farms

BTM ELISA	Number of farms	Average number of positive animals per farm	Herd prevalence	Number of serological positive goats per farm out of 13					
				0	1	2	3	4	≥5
Negative	46	0.70	5.4%	35	6	1	1	0	3
Low positive	8	2.25	17.3%	2	0	3	1	1	1
Positive	22	5.59	43.0%	0	2	2	2	2	14
High positive	1	4.00	30.8%	0	0	0	0	1	0
Total	77	2.30	17.7%	37	8	6	4	4	18

BTM Bulk tank milk

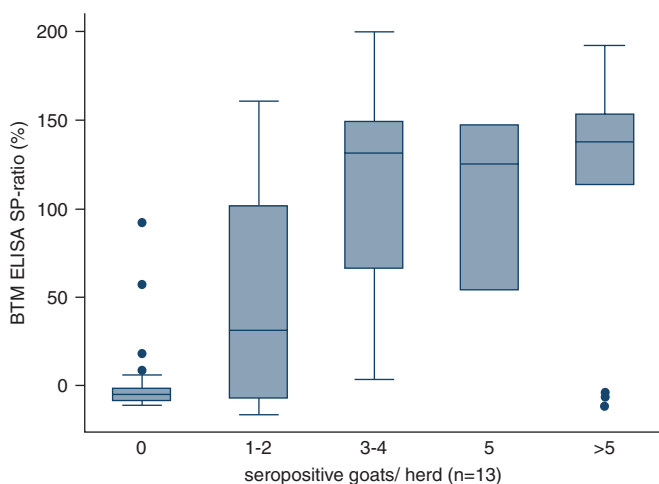


FIG 4: Comparison of the BTM ELISA S/P ratio and the number of seropositive animals, from 13 animals sampled, serologically tested by ELISA, per herd. Note that the results of many farms are zero for both within-herd seroprevalence and ELISA SP-ratio

[95 per cent CI: 27.8 to 30.4]) were BTM PCR positive ($P < 0.001$), and 70 (25.5 per cent [95 per cent CI: 24.3 to 26.7]) were BTM ELISA positive ($P < 0.001$). Although the time period between abortion and BTM sampling differed from zero to three years, both ELISA and PCR results did not change by year of abortion.

Q fever prevalence in BTM (PCR, ELISA) and individual samples (ELISA)

A total of 308 BTM samples from dairy sheep and dairy goat farms were tested by RT-PCR and ELISA, and 1053 serum samples from 81 dairy sheep and dairy goat farms were tested for antibodies against *C burnetii*. From these 1053 samples, 181 (17.2 per cent [95 per cent CI: 14.9 to 19.5]) were serological positive.

Between 2005 and 2008, IHC-confirmed Q fever abortion was diagnosed on 21 dairy goat and two dairy sheep farms before BTM sampling in 2008. One dairy goat farm had an outbreak of Q fever abortion after submitting the BTM sample and classified as having no history of Q fever abortion at the time of BTM sampling.

Dairy sheep

A total of 16 out of 40 dairy sheep farms submitted a BTM sample. None was PCR positive and three (18.8 per cent [95 per cent CI: 4.0

to 33.6]) were ELISA positive for *C burnetii*.

From four dairy sheep farms, which were all BTM ELISA and PCR negative, in total 52 serum samples were available. Four serum samples (7.7 per cent [95 per cent CI: 0.0 to 14.9]) originating from one farm, were seropositive. On this farm, no abortion caused by *C burnetii* was notified. Two dairy sheep farms had a history of abortion waves caused by *C burnetii*. One of these two farms submitted a BTM sample. This BTM sample was ELISA positive and PCR negative.

Dairy goats

A total of 292 out of 392 dairy goat farms submitted BTM samples, of which 158 (54.1 per cent) originated from farms that were situated in the south eastern provinces of The Netherlands. 96 BTM samples (32.9 per cent

[95 per cent CI: 30.2 to 35.6]) were PCR positive (Table 1). 75 (78.1 per cent [95 per cent CI: 69.9 to 86.4]) from the 96 BTM PCR positive goat farms were situated in the south eastern provinces of The Netherlands. In this region, 50.2 per cent (95 per cent CI: 46.5 to 53.9) of the investigated BTM samples was PCR positive, compared with 15.7 (95 to CI: 12.6 to 18.8; $P < 0.001$) in the other provinces of The Netherlands.

87 (29.8 per cent [95 per cent CI: 27.2 to 32.5]) BTM samples were ELISA positive (Table 1). Of this, 68 were situated in the south eastern provinces. In this region, 43 per cent (95 per cent CI: 39.3 to 46.7) of the BTM samples was ELISA positive, compared with 15.7 per cent (95 per cent CI: 12.6 to 18.8; $P < 0.001$) in the other provinces of The Netherlands.

From 77 dairy goat farms, 1001 individual serum samples were available and the average seroprevalence on these farms was 17.7 per cent (95 per cent CI: 15.3 to 20.0). The within-herd seroprevalences were 42.3 per cent, 39.4 per cent, 9.0 per cent and 6.2 per cent on BTM PCR very high positive, high positive, weak positive and negative farms, respectively (Table 2). The within-herd seroprevalences differed significantly between BTM PCR (very) high positive farms and BTM PCR weak positive farms (Fig 3). No significant difference in within-herd seroprevalence was found between BTM PCR weak positive farms and negative farms.

The within-herd seroprevalences were 30.8 per cent, 43.0 per cent, 17.3 per cent and 5.4 per cent on BTM ELISA high positive, positive, low positive and negative farms, respectively (Table 3). The within-herd seroprevalences differed significantly between BTM ELISA high positive, positive, and low positive farms on the one hand, compared with BTM ELISA negative farms on the other hand (Fig 4).

From the 292 BTM samples, 17 were obtained from farms with a confirmed history of Q fever abortion. From these 17 farms, 16 (94.0 per cent) were BTM PCR positive and all of them were BTM ELISA positive.

Discussion

The first aim of this study was to determine the agreement between the results of a commercially available ELISA and RT-PCR in the same BTM samples and individual serum samples from dairy goat and dairy sheep farms with and without a history of IHC-confirmed Q fever abortions. Therefore, the BTM PCR and ELISA test results were compared with each other and with the within-herd seroprevalences as determined by individual serology. Since Muskens and others (2011) showed a correlation between PCR prevalence and ELISA prevalence in herds, it was calculated at what PCR level the correlation between BTM PCR and ELISA results was the maximum. It appeared that for

the chosen cut-off levels of the PCR, the cut-off level of 100 bacteria/ml results in the highest area under the ROC curve. This cut-off level is the same as the cut-off level that is recommended by the manufacturer as cut-off between weak- and high positive results. Using this cut-off, the proportion of agreement is at most at ELISA S/P ratio of 93 per cent, which is near the cut-off level of 100 which is recommended by the manufacturer, giving a sensitivity of 88.2 per cent and a specificity of 94.6 per cent. Therefore, the cut-off levels as given by the manufacturer are used for the descriptive part of the study. Taking into account that there is no real gold standard for quantifying *C burnetii* on BTM level and PCR and ELISA are based on different principles, the agreement between PCR and ELISA results in BTM is sufficient. In the absence of a gold standard or reference value, both sensitivity and specificity are relative. In addition to comparison with PCR results, BTM ELISA results were also compared with individual seroprevalences. The correlation between BTM ELISA results and within-herd seroprevalences was highest at a seroprevalence cut-off of 15 per cent and a BTM ELISA cut-off of 46 per cent S/P-ratio. For these criteria, the specificity and sensitivity were 91.0 per cent and 84.3 per cent, respectively, which is sufficient for large-scale monitoring. Under these conditions, the BTM ELISA has a lower sensitivity than reported in a recent study for cattle in The Netherlands but a much higher specificity (Muskens and others 2011). However, the latter used a cut-off for ELISA of 30 per cent and prevalence cut-off of 10 per cent.

In this study, PCR and ELISA results are coherent when the tests are not used for finding the last positive animal but for detecting within-herd prevalences of 15 per cent or more, which is only slightly different from findings in cattle herds in a recent study in The Netherlands with the same tests (Muskens and others 2011).

The second aim of this study was to describe the Q fever prevalence on farm level by testing BTM samples using the above-mentioned ELISA and RT-PCR, related to the results of individual blood samples and of IHC-confirmed Q fever abortions.

None of the dairy sheep BTM samples was PCR positive. This was also found in Switzerland (Fretz and others 2007) but differs from a study in the Basque Country where 22 per cent of the sheep flocks tested positive by PCR (García-Pérez and others 2009). However, results from different countries are difficult to compare, both as a result of different test protocols and different epidemiological circumstances (Guatteo and others 2011). It might be that in the present study, the bacterium was totally absent on the dairy sheep farms, at the time of sampling, but it could also be that the bacterium resided in other matrices than milk (Rodolakis and others 2007, Astobiza and others 2011). Since infected sheep mainly shed *C burnetii* in milk during a short period after parturition, sampling shortly after lambing might have led to higher prevalences (Rodolakis and others 2007, Roest and others 2011).

BTM samples were submitted by 292 (76.2 per cent of all) Dutch dairy goat farms in 2008, and 96 (32.8 per cent) BTM samples were PCR positive. This percentage is higher than found in Switzerland, where none of the 39 BTM samples from goat farms was PCR positive (Fretz and others 2007). In Iran, only 1 of 56 BTM samples from 20 goat breeding farms was PCR positive (Rahimi and others 2010). In the south eastern provinces of The Netherlands, significantly more dairy goat BTM samples (50.2 per cent) were PCR positive compared with the remaining provinces (15.7 per cent). The within-herd seroprevalence of farms with very high positive or positive BTM PCR results were 39.4 per cent and 42.3 per cent, respectively. For farms with PCR BTM negative or weak positive results, the within-herd seroprevalence was 6.2 per cent and 9.0 per cent, respectively, which was significantly lower. No significant differences in within-herd seroprevalences were found between PCR weak positive farms and PCR negative farms, indicating that the cut-off value of the RT-PCR of 100 bacteria/ml, as given by the manufacturer, is a reliable indication of the infection status of the herd or flock. Analysing the feasibility of the PCR for dairy goat BTM samples, in this study, the area under the curve was highest at a cut-off value of 100 bacteria/ml, which would therefore be the preferred cut-off.

BTM samples were also tested for antibodies. Until now no studies have been published describing the diagnostic performance of a Q fever BTM ELISA for large numbers of dairy goat farms. From 292 of

all BTM samples, 87 (29.8 per cent) were ELISA positive. In the south eastern provinces of The Netherlands, significantly more farms were ELISA positive compared with the other provinces which is in line with the BTM PCR results. A clear correlation between within-herd seroprevalences and ELISA BTM results was found; within-herd seroprevalences were significantly higher on ELISA BTM positive farms (43.0 per cent) than on ELISA BTM negative farms (5.4 per cent). Unexpectedly, on three BTM ELISA and BTM PCR negative farms 6, 6 and 9 seropositive animals were found, respectively. This might be caused by unintended biased sampling. A negative BTM PCR combined with high within-herd seroprevalences may also have been caused by former *C burnetii* infections without current shedding. It is useful to continue monitoring on these farms during a longer period.

In this study, by BTM testing, all 17 farms with an IHC-confirmed *C burnetii* abortion outbreak were detected by ELISA and one was missed by PCR which could be explained by the interval between abortion and testing. When, on the contrary, only the IHC-confirmed farms were regarded as true positives, both PCR and ELISA were largely lacking specificity.

PCR testing of BTM samples has some limitations: a single BTM PCR test result only gives information about shedding in milk at one particular moment. A positive BTM PCR can be caused by only a few shedding animals, and shedding via other routes (Rodolakis and others 2007) is not determined in this way. However, the results of this study demonstrate a clear correlation between BTM PCR and ELISA and individual serology. BTM testing is a proper tool for Q fever monitoring purposes in dairy goats.

Acknowledgements

This study was financially supported by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV). The authors would like to thank Willem Wouda for his comments on the description of the performance of the IHC on aborted placentas.

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Veterinary Record 2012 170: 310 originally published online February 20, 2012
doi: 10.1136/vr.100304

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