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

R. van den Brom, E. van Engelen, S. Luttikholt, L. Moll, K. van Maanen, P. Vellema

Context
In 2007, a human epidemic of Q fever with a suspected relationship to dairy goats started in the south-eastern region of the Netherlands. As a precaution, the Dutch government decided to implement measures on infected dairy sheep and goat farms. It was therefore necessary to distinguish between infected and non-infected farms. To detect Coxiella burnetii infection, tests for individual animals are available. Taking into account the size of Dutch dairy goat farms, with an average of 900 adult animals per herd, a monitoring programme based on repeated testing of individuals would be expensive and difficult to perform, and so bulk tank milk (BTM) testing might be a useful alternative. BTM sampling on dairy cattle farms has already shown its value for several agents. The aim of this study was, first, to determine the agreement between the results of a commercially available ELISA and real-time PCR in BTM samples and individual serum samples from dairy goat and dairy sheep farms with and without a history of abortions associated with Q fever; secondly, to describe the prevalence of C. burnetii at farm level by testing BTM samples using this ELISA and real-time PCR, related to the results of individual blood samples and of immunohistochemistry (IHC)-confirmed Q fever abortions.

Main conclusion
The agreement of the BTM PCR and ELISA on the one hand, and the test results of individual serum samples and a history of confirmed Q fever abortion on the other, is sufficient for BTM PCR and ELISA to be used for monitoring purposes. In the south-eastern region of the Netherlands, which experienced a large human Q fever outbreak in 2007, a significantly larger proportion of the BTM samples was PCR- and ELISA-positive compared with samples from the rest of the country. This supports the suspected relationship between human cases of Q fever and infected dairy goat farms.

Approach
In 2008, all 392 dairy goat farmers and 40 dairy sheep farmers in the Netherlands were asked for a BTM sample to be tested for C. burnetii using a commercial ELISA and a real-time PCR. Serum samples were submitted from randomly selected farms as part of the annual Brucella melitensis monitoring programme and were tested by ELISA. The BTM results of farms with an IHC-confirmed history of Q fever abortion were compared with the BTM results of farms without a notified history of Q fever abortion. The BTM results were also used to determine the test characteristics. Log-transformed quantitative PCR data were compared with ELISA sample-positive (S/P) ratios. Different cut-off levels of the BTM PCR were taken as reference values, and for each PCR cut-off level ROC curves were plotted for the different BTM ELISA S/P ratios. This was also done for different prevalence cut-off levels.

Results
A total of 308 BTM samples from dairy sheep and dairy goat farms were tested by real-time PCR and ELISA. From the 292 goat BTM samples, 29.8 per cent (95 per cent confidence interval [CI] 27.2 to 32.5) were ELISA-positive and 32.9 per cent (95 per cent CI 30.2 to 35.6) were PCR-positive. The overall percentage of seropositive goats was 17.7 per cent (n=1001). From 77 herds, 51.9 per cent (95 per cent CI 41.9 to 61.9) of the herds contained one or more positive animals out of 13 sampled animals. From these herds with positive samples, a mean of 4.4 and a median of four animals out of 13 were positive. All farms with a history of Q fever abortion (n=17) were BTM ELISA-positive, and 16 of 17 were also PCR-positive. BTM PCR- or ELISA-positive farms had significantly higher within-herd seroprevalences than BTM-negative farms. When compared with the ELISA results, the optimal cut-off value for the BTM real-time PCR was 100 bacteria/ml. From the 16 sheep BTM samples, 18.8 per cent (95 per cent CI 4.0 to 33.6) were ELISA-positive and none was PCR-positive.

BTM log-transformed quantitative PCR data and ELISA S/P ratios had a correlation coefficient of 0.90. For 13 different chosen PCR cut-off levels, the highest level was reached at the ROC curve of the ELISA was 0.968, and was reached at a PCR cut-off of 100 bacteria/ml. The maximum proportion of agreement was reached at an ELISA cut-off of 93 per cent S/P ratio, with 88.2 per cent sensitivity and 94.6 per cent specificity. At ELISA cut-off levels of 30, 100 and 200 S/P ratios, as indicated by the manufacturer, the sensitivity was 95.6, 85.3 and 8.2 per cent, and the specificity was 89.6, 95.0 and 99.6 per cent, respectively. When the BTM ELISA results were compared with chosen cut-off levels of seroprevalences (8, 15, 23, 46 and 62 per cent) for the ELISA, the area under the ROC curve was highest (0.8774) using a within-herd seroprevalence of 15 per cent. In that situation, the proportion of agreement was highest (88.3 per cent) at a BTM ELISA cut-off of 46 per cent S/P ratio, with a specificity of 84.3 per cent and specificity of 91.1 per cent. The correlation coefficient between within-herd seroprevalences and BTM ELISA S/P ratio was 0.72.

Interpretation and notes of caution
Taking into account that there is no real gold standard for quantifying C. burnetii in BTM and that PCR and ELISA techniques are based on different principles, the agreement between the PCR and ELISA results in BTM is sufficient. However, in the absence of a gold standard, both sensitivity and specificity are relative. In addition, the value of BTM PCR testing is limited and gives information only about a particular animal but for detecting within-herd seroprevalences and BTM ELISA S/P ratio was 0.90. For 13 different chosen PCR cut-off levels, the highest level was reached at the ROC curve of the ELISA was 0.968, and was reached at a PCR cut-off of 100 bacteria/ml. The maximum proportion of agreement was reached at an ELISA cut-off of 93 per cent S/P ratio, with 88.2 per cent sensitivity and 94.6 per cent specificity. At ELISA cut-off levels of 30, 100 and 200 S/P ratios, as indicated by the manufacturer, the sensitivity was 95.6, 85.3 and 8.2 per cent, and the specificity was 89.6, 95.0 and 99.6 per cent, respectively. When the BTM ELISA results were compared with chosen cut-off levels of seroprevalences (8, 15, 23, 46 and 62 per cent) for the ELISA, the area under the ROC curve was highest (0.8774) using a within-herd seroprevalence of 15 per cent. In that situation, the proportion of agreement was highest (88.3 per cent) at a BTM ELISA cut-off of 46 per cent S/P ratio, with a specificity of 84.3 per cent and specificity of 91.1 per cent. The correlation coefficient between within-herd seroprevalences and BTM ELISA S/P ratio was 0.72.

Significance of findings
The results of this study demonstrate a clear correlation between BTM PCR and ELISA results and individual serology. BTM testing could therefore be useful as a tool for the purpose of monitoring Q fever in dairy goats.
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