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Bayesian estimation of sensitivity and specificity of *Coxiella burnetii* antibody ELISA tests in bovine blood and milk

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ABSTRACT

Serological tests for *Coxiella burnetii* (the causative agent of Q fever) antibodies are usually based on enzyme linked immunosorbent assay (ELISA) although this method is not thoroughly evaluated. The objective of this study was to determine the sensitivity and specificity of an ELISA for detection of *C. burnetii* antibodies in milk and blood samples, using latent class models in a Bayesian analysis. Blood and milk samples of 568 lactating cows from 17 Danish dairy cattle herds collected in 2008 were used.

The best combination of sensitivity and specificity estimates was revealed at a sample to positive (S/P) cut-off of 40 for both blood and milk ELISAs. At this cut-off, sensitivity of milk ELISA was 0.86 (95% posterior credibility interval [PCI] [0.76; 0.96]). This was slightly but insignificantly higher than sensitivity of blood ELISA (0.84; 95% PCI [0.75; 0.93]). The specificity estimates of the ELISA methods on milk and blood were equal at 0.99. No conditional dependence was observed between the specificity estimates of the two test methods. However, the sensitivity estimates of both tests were significantly reduced when conditional covariances ≥40 were used. Collection of milk samples from lactating cows is relatively easy, non-invasive and inexpensive and hence milk ELISA may be a better option for screening lactating cows. But, blood ELISA is an option for screening non-lactating cattle.

1. Introduction

Diagnosis of *Coxiella burnetii* infection (Q fever) in animals depends on detection of bacteria, bacterial components or antibodies (Bouvery et al., 2003; Fournier et al., 1998; Rodolakis, 2006). Detection of bacteria can be done by culturing, but usually detection of bacterial components, e.g. by PCR is used (Rodolakis, 2006). Although PCR is often considered the most appropriate technique for *C. burnetii* detection, it is an expensive and time consuming procedure and it depends on the actual presence of bacterial DNA (Rousset et al., 2010). Therefore, cheap and fast serological techniques are still widely used as screening tests and in large scale epidemiological studies. However, serological tests can only identify the antibody response to infection. CFT and ELISA are the two most commonly used serological techniques for screening in animals for *C. burnetii* exposure (Rousset et al., 2010). Nowadays, ELISA is becoming more popular than CFT for its better reliability and handiness (Rousset et al., 2010) and a recommended test for Q fever diagnosis in the European Union (EFSA Panel on Animal Health and Welfare (AHAW), 2010). ELISA can be used to detect antibodies both in milk and in blood samples. In recent years, milk analyses have been widely used in epidemiological studies involving dairy cattle since they have some advantages over blood analyses. Milk samples are easier and less expensive to collect, and non-invasive and hence minimize stress to the cow. However, blood ELISA is still necessary for the studies involving non-lactating cattle.

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The performance of a diagnostic serological test can be evaluated against a perfect test with 100% sensitivity and 100% specificity (gold standard). However, none of the existing *C. burnetii* serological tests are perfect, as also known for diagnostic tests of most other infections (Rousset et al., 2010). Performance of the *C. burnetii* antibody ELISA has been evaluated in few studies based on the performance of CFT, which gave relative estimates of sensitivity and specificity (Emery et al., 2012; Kittelberger et al., 2009). In situations where the case definition is determined by an imperfect reference test, selection bias and/or misclassification bias might be introduced (Nielsen and Toft, 2002). In a situation where a reference test is unavailable, latent class analysis (LCA) is an alternative option for estimating sensitivity and specificity of two or more tests without assuming the underlying true antibody status of test subjects, which eliminates the chance of selection bias in the estimates. LCA can be done either by fitting the model using maximum likelihood procedures or by Bayesian analysis (Enøe et al., 2000). In LCA, three assumptions known as the Hui–Walter paradigm are generally made: (1) at least two populations with different prevalences are required, (2) the sensitivity and specificity of the tests do not differ across the populations, and (3) the tests under evaluation are conditionally independent given the disease status (Hui and Walter, 1980). However, especially for the tests based on the same biological principle, in our case detection of antibodies, the assumption about conditional independence given disease status can be questioned. In a recent study the performance of CFT and three commercially available ELISAs were evaluated in a LCA framework by fitting the model using maximum likelihood estimation (Horigan et al., 2011). In that study all the tests were assumed to be conditionally independent. This assumption of conditional independence influences the test properties. If a positive correlation between the tests under evaluation is ignored it will lead to overestimation of the test performance, while ignorance of a negative correlation will cause underestimation of the test performance (Vacek, 1985).

The objective of this study was to estimate the sensitivity and specificity of milk ELISA and blood ELISA for detection of *C. burnetii* antibodies accounting for the conditional dependence between the two test methods, using latent class models in a Bayesian analysis in a population of spontaneously infected cows from selected Danish dairy cattle herds.

2. Materials and methods

2.1. Sample collection and population stratification

The data included 568 lactating cows from 17 Danish dairy cattle herds. The herds were recruited from 100 dairy herds that were randomly selected among 4785 milk delivering herds in the spring 2008 and examined for the level of *C. burnetii* antibodies in a bulk tank milk (BTM) sample (Agger et al., 2010). The 100 herds were stratified into positive, negative and intermediate herds based on the level of *C. burnetii* antibodies (expressed as S/P value) in a single BTM sample. Based on the cut-off recommended by the manufacturer, a herd with S/P $\geq$ 40 was considered positive and a herd with S/P < 30 was negative, whereas a herd with S/P 30–39 was classified as intermediate. Following this initial study, eight positive, six negative and three intermediate herds were selected by systematic random sampling within the three strata. These three groups were used to form the subpopulations with assumed differences in animal-level prevalences. Milk of all lactating cows within the selected herds were sampled. However, only 568 of these were blood sampled due to the economics of the project. Only cows with both sample types were included in this study, i.e. 568 cows. The cows to be blood sampled were selected by a within herd systematic random sampling procedure. Milk sampling was done by the farmers as part of the milk control scheme and the blood samples were taken by the herd veterinarians. The time interval between the milk and blood sampling was minimized and ranged from zero to three days with median one. All samples were collected between August 2008 and October 2008 and sent to the National Veterinary Institute, Technical University of Denmark maintaining standard cool chain and tested by ELISA according to instructions by the test kit manufacturer.

2.2. Diagnostic tests

2.2.1. Milk ELISA

Ten milliliter milk from each cow was collected for testing. The fat fraction was removed from the milk by centrifugation and the non-fat fraction was stored at −20 °C until tested for antibodies against *C. burnetii* using the commercial CHEKIT Q-Fever Antibody ELISA Test Kit (IDEXX, Liebefeld-Bern, Switzerland) based on *C. burnetii* inactivated phase 1 and phase 2 antigens. All samples were tested in duplicates and the optical densities (OD) of the samples were averaged and corrected by subtracting the OD of the negative control. The results were expressed as S/P values and estimated using equation recommended by the manufacturer (Agger et al., 2010; Paul et al., 2012). The S/P values were estimated on a continuous scale with a theoretical range from zero to "plus infinity". The manufacturer of the test kit recommends a S/P cut-off $\geq$40 for classifying test subjects as positive. At this cut-off we considered a sample with S/P < 40 as negative. However, according to our study objective we investigated the test results according to three different S/P cut-offs where S/P values $\geq$30, $\geq$40 and $\geq$50, respectively were considered as test positive, and S/P values $\geq$30, $\geq$40 and $\geq$50 were considered test negative respectively.

2.2.2. Blood ELISA

Five to eight milliliter blood from each selected cow was collected. Upon arrival at the laboratory, samples were centrifuged at 3000 × *g* for 10 min for serum separation. Sera were stored at 5 °C and tested for antibodies against *C. burnetii*. The same ELISA kit and laboratory procedure was used for milk and blood samples and the results were expressed as S/P values with cut-off values as mentioned above.