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A probabilistic approach to the interpretation of milk antibody results for diagnosis of Johne's disease in dairy cattle

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ABSTRACT

Johne's disease is a serious wasting disease of ruminants that is of high economic importance for the dairy sector in particular. The chronic nature of the disease, the fluctuations in antibody levels and the limited ability of diagnostic tests to identify cows at early stages of infection are huge challenges for the control of the disease. In the United Kingdom, the latter is commonly based on repeated milk ELISA testing of lactating cows, followed by selected culling and improved management practices around calving. In this paper, the dataset built through a large quarterly screening programme conducted in the United Kingdom since 2010 is used to investigate the use of milk ELISA testing for Johne's disease management. Over the study period, 13,509 out of 281,558 cows were identified as high-risk of being infected and shedding mycobacteria in the faeces, based on a case definition of at least two consecutive positive milk ELISA results. Around a third of them were kept in the dairy herd a year or more after being classified as high-risk. However, 16% of these cows did not have any further positive test, suggesting that they might be uninfected animals. The mean specificity and sensitivity of the milk ELISA test were estimated at 99.5% and 61.8%, respectively. The cows in the dataset are categorised in different result groups according to the number of positive test results and whether they are classified as high-risk according to the programme's case definition. The posterior probability of infection is calculated after each test in order to investigate the impact of repeated testing on the belief in a cow's infection status. The interpretation of the results show that most cows classified as high-risk are very likely to be infected, while some other groups that do not match the case definition could reasonably be considered as infected too. Our results show that there is considerable potential for more targeted use of serological testing, including adjusting the testing frequency and implementing the posterior probability approach.

1. Introduction

Johne's disease (JD),[1] caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a disease of serious economic importance for the dairy sector (Harris and Barletta, 2001; Ott et al., 1999; Stott et al., 2005). However, reliable impact estimates require precise data on the prevalence of the disease, which have been elusive largely due to the chronic nature of the disease and the limited ability of diagnostic tests to identify cows at early stages of infection. Nielsen and Toft in a systematic review carried out in 2009 concluded that accurate prevalence estimates were largely lacking across European countries (Nielsen and Toft, 2009). In the United Kingdom (UK), a study conducted in the late 90 s reported an animal-level prevalence of infection of between 2.6 and 3.5% in Southwest England (Çetinkaya et al., 1996). Another study based on postal surveys reported a prevalence of between 17% and 71% of clinically infected herds in different study areas across the UK (Cetinkaya et al., 1998; Daniels et al., 2002). In a later study, it was estimated that 75–78% of herds in Southwest England had at least one seropositive animal (Woodbine et al., 2009). More recently, Velasova et al. (2017) estimated the herd level prevalence of JD across Great Britain as 68% (95% confidence interval: 59–77%) by means of antibody detection in bulk milk samples. Poor sensitivity of the available ante-mortem diagnostic tests poses a challenge for accurate estimation of JD prevalence and farm-level decision making with regard to

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[1] *Abbreviations used:* ELISA, enzyme-linked immunosorbent assay; JD, Johne's disease; MAP, *Mycobacterium avium* subsp. *paratuberculosis*; MES, mean effective sensitivity; NMR, National Milk Record group; PPI, posterior probability of infection; S/P, sample-to-positive ratio; UK, United Kingdom.

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individual cows (Collins et al., 2006; Eamens et al., 2000; Nielsen and Toft, 2008). Among these tests, enzyme-linked immunosorbent assays (ELISA) for the detection of antibodies in milk are commonly used due to performance, convenience and cost (Nielsen and Toft, 2008). The ability of these tests to correctly identify JD-infected cows is highly dependent on their age and stage of infection (Hanks et al., 2013; Nielsen et al., 2002a,b; Nielsen et al., 2013; Sweeney et al., 2006; van Schaik et al., 2003). Test sensitivity (the proportion of infected cows detected as positive by the test) is low at early stages of infection and increases as the disease progresses (Nielsen et al., 2013). For this reason, decisions regarding the management of individual cows are normally made upon consideration of the results of repeated tests together with either clinical manifestations and/or other factors such as somatic cell count, milk yield and fertility. To achieve reduction of the prevalence in the herd, it is commonly recommended to farmers to cull cows repeatedly testing positive, as they are more likely to be excreting MAP into the environment (Nielsen et al., 2002b; Nielsen, 2008; Sweeney et al., 2006) and to have reduced milk yield (Nielsen et al., 2009). A number of studies have formally investigated the control of JD in dairy herds and its impact, especially in the United States (Aly et al., 2012; Robins et al., 2015; Smith et al., 2017) and in Denmark (Kudahl et al., 2008, 2007). However, only one study has focused on JD control in the UK dairy sector so far (Stott et al., 2005), and it only looked at the impact of decreased milk yield and early culling. In the UK, JD control is usually based on repeated testing of all cows in infected herds associated with selective culling and improved calving and calf management practices. Anecdotal evidence suggests that the course of action following a specific set of test results is highly inconsistent. Therefore, individual cow management could be an area where farmers and veterinarians would benefit from further evidence to inform their decisions. In this study, a large dataset of individual cow milk ELISA test results was used to investigate several critical aspects in relation to the use of repeated tests for the purpose of JD management. The primary objective of the study was to evaluate whether using a probabilistic approach could support interpretation of repeated test results for individual cows. To this effect, we firstly estimated the test performances (sensitivity and specificity) based on the test results obtained for cows at different ages. Then, we calculated the true within-herd seroprevalence and estimated the predictive values of single and repeated testing. These data were combined to calculate and analyse the posterior probability of infection of individual cows after each test result. Lastly, we assessed the potential implications of our results on the current recommendations for management and culling of cows deemed as “high-risk”.

2. Material and methods

2.1. Source of data and data management

The study was based on detailed exploration of a dataset provided by the National Milk Records Group (NMR) containing 1,694,172 complete records representing all JD milk ELISA tests conducted by NMR from 01/01/2010 to 18/05/2015. ELISA test results included in the dataset were performed within a JD screening programme for dairy herds (‘HerdWise’) provided by the NMR group in the UK. Within herds enrolled in this programme, all milking cows are tested for JD antibodies by milk ELISA on a quarterly basis using samples obtained for milk recording. A single herd can therefore contribute records from multiple cows and an individual cow can be represented in the dataset with multiple records, representing tests conducted at different points in time. Each record is one milk ELISA test result, with the following information: identification of the herd and the cow, birth date of the cow, date of the test and optical density value obtained from the sample. Milk samples are collected during milking, into pots containing bronopol as a preservative and delivered to the laboratory within 48 h of collection. The samples are de-fatted and tested by means of a commercial ELISA test. The same test has been in use over the entire period (IDEXX Paratuberculosis Screening Ab Test, IDEXX Laboratories, Maine, United States). The test interpretation was performed following the manufacturer’s instructions using a cut-off of 30: tests with a sample-to-positive ratio (S/P) of 30 or above were considered positive, whilst others were considered negative. Within the HerdWise programme, the so-called “red cows” are defined as cows with two consecutive positive milk ELISA results and as a result are deemed to be at high-risk of being infected and shedding MAP in the faeces.

2.2. Evaluation of single test performance

We estimated the age-specific specificity and sensitivity of the milk ELISA test from the available data, following the approach described by Nielsen et al. (2013). The target condition was defined as MAP-infected cow in which a humoral response would become detectable within the economic life of the cow. The transition from a cell-mediated response to a detectable humoral response has been associated with the progression of the disease, development of symptoms and faecal bacterial shedding (Koets et al., 2015; Nielsen et al., 2009; Stabel, 2000). Each cow from the dataset was classified as case or non-case for this condition according to the definitions presented below, or excluded from the dataset if it did not comply with either definition.

To evaluate the specificity of a single test, cows with at least nine test results and for which the last eight tests were negative were classified as non-cases. Non-cases were considered as non-MAP infected or as MAP infected but with no progression of the infection during the cow’s lifetime (Mitchell et al., 2015). Eight negative tests correspond to around two years of negative tests in a quarterly programme. Preliminary data analysis showed that most of the cows with at least one positive test had seroconverted by the age of five. As testing starts around two to three years of age, this definition enabled us to retain cows that were likely to be non-MAP infected. Based on this definition, 55,586 cows were selected as non-cases. To avoid dealing with correlated data, one test result from each non-case cow (and the age at which it was obtained) was selected at random. The specificity of the test was estimated as the number of negative tests among the tests obtained from non-cases. The lower and upper limits of the confidence interval of the specificity were calculated with the Agresti-Coull method which is recommended for large samples (Brown et al., 2001).

To evaluate the sensitivity of a single test, we chose a case definition matching the “red cow” definition used within the HerdWise programme. Cows with at least three test results available and where the two last tests conducted yielded a positive result were classified as cases. Based on this definition, 9553 cows were selected as cases, and, therefore, as MAP-infected cows with progression of the infection. One test result from each case cow (and the age at which it was obtained) was selected at random. The age-specific sensitivity *Se(t)* of the test at age *t* is the proportion of positive tests at a given age among the tests obtained from cases. *Se(t)* was estimated using a non-linear logistic regression model (Nielsen et al., 2013):

$$\text{logit}(Se(t)) = a - b^{*}e^{-c^{*}t}$$

where *a* is the upper limit of the logit function when *t* increases, *b* is a scaling factor and *c* is the coefficient for the decay of the age effect. The inverse-logit of *a* is the upper limit of the age-specific sensitivity when age increases. The age-specific sensitivity accounts for both the probability that an infected cow excretes detectable levels of MAP antibodies in the milk and the intrinsic characteristics of the milk ELISA. As age increases, the probability that an infected cow secretes detectable levels of MAP antibodies in the milk increases. Therefore, the upper limit of the age-specific sensitivity is a good estimator of the sensitivity of the ELISA test in an infected cow which is secreting detectable level MAP antibodies in its milk at the time of testing. Parameters *b* and *c* condition the rate at which the sensitivity increases with age. After the model was fitted, the Pearson’s correlation coefficient between