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The effect of alternative testing strategies and bio-exclusion practices on Johne's disease risk in test-negative herds

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ABSTRACT

Herd classification is a key component of national Johne's disease (JD) control programs. Herds are categorized on the basis of test results, and separate subprograms are followed for test-positive and test-negative herds. However, a test-negative herd result does not necessarily equate to JD freedom for reasons relating to disease pathogenesis and available diagnostic tests. Thus, in several countries, JD control programs define test-negative herds as having a "low risk" of infection below a specified prevalence. However, the approach is qualitative, and little quantitative work is available on herd-level estimates of probability of freedom in test-negative herds. This paper examines the effect over time of alternative testing strategies and bio-exclusion practices on JD risk in test-negative herds. A simulation model was developed in the programming language R. Key model inputs included sensitivity and specificity estimates for 3 individual animal diagnostic tests (serum ELISA, milk ELISA, and fecal culture), design prevalence, testing options, and testing costs. Key model outputs included the probability that infection will be detected if present at the design prevalence or greater (herd sensitivity; *SeH*), the probability that infection in the herd is either absent or at very low prevalence (i.e., less than the design prevalence; *ProbF*), the probability of an uninfected herd producing a false-positive result [*P*(*False+*)], and mean testing cost (*HerdCost*) for different testing strategies. The output *ProbF* can be updated periodically, incorporating data from additional herd testing and information on cattle purchases, and could form the basis for an output-based approach to herd classification. A high *ProbF* is very difficult to achieve, reflecting the low sensitivity of the evaluated tests. Moreover, *ProbF* is greatly affected by any risk of introduction of infection, decreasing in herds with poor bio-exclusion practices despite ongoing negative test results. The value of *P*(*False+*) was substantial when tests with imperfect specificity were used. Testing strategies can substantially influence testing costs but with little effect on test performance. This study illustrates an output-based approach to herd classification, with potential for national and field applications.
Key words: Johne's disease, testing strategy, bio-exclusion, probability of freedom

INTRODUCTION

Herd classification is a key component of national Johne's disease (**JD**) control programs, enabling commerce to continue while protecting herds with low infection risk. Herds are categorized on the basis of test results, and separate sub-programs are followed for test-positive and test-negative herds (Tavornpanich et al., 2012). In herds with test-negative results, herd owners seek to minimize the risk of introduction of *Mycobacterium avium* ssp. *paratuberculosis* (**Map**, the causative agent of JD) through attention to bio-exclusion and to build confidence of JD freedom. However, JD freedom among test-negative herds is very difficult to prove because of the prolonged incubation period and the poor individual animal sensitivity (**Se**) of available diagnostic tests, especially during the early stages of the disease. Animals most commonly acquire Map infection during the early stages of life (Whittington and Windsor, 2009; Windsor and Whittington, 2010; Lombard, 2011). Time between onset of infection to detectable fecal shedding is variable but usually >2 yr, and animals can be infectious, and thus shedding Map, for a variable period before progressing to clinical disease (Sweeney, 2011). Therefore, animals can be infected, and infectious, for extended periods before developing clinical signs (Nielsen and Toft, 2008).

Several countries have developed programs for test-negative herds, cognizant of the above-mentioned con-

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cerns, to enable cattle herd owners to objectively and transparently demonstrate a specified low risk of Map infection, including the Market Assurance Program in Australia (**CattleMAP**; Animal Health Australia, 2008) and the Voluntary Herd Status Program in the United States (United States Department of Agriculture, 2010). In CattleMAP, movement to higher herd assurance (classification) levels [monitored negative (**MN**)1 to MN2 to MN3] relies on ongoing test-negative results at, generally biennial, intervals while complying with defined testing regimens and rigorous herd biosecurity requirements. Detailed program rules are in place (Animal Health Australia, 2008).

As yet, limited quantitative work has been done on herd-level estimates of probability of freedom in test-negative herds. Sergeant et al. (2008) investigated the probability of low JD prevalence in Danish dairy herds, but only at a single point in time. The rules in the above-mentioned Australian and US programs are essentially based on qualitative assessments, and there is currently no understanding of the relative difference in probability of freedom between herds at different assurance (classification) levels, such as MN1 versus MN2. A quantitative assessment would be useful, both to provide numeric estimates of probability of freedom and to identify the factors that are most influential in increasing, or decreasing, these estimates over time. Rapid progress has been made in quantitative methods to substantiate freedom from infection, most recently using scenario trees (Martin et al., 2007a). To date, these methods have primarily been used at a regional or national level to substantiate freedom from a range of infectious diseases (Martin et al., 2007b; More et al., 2009; Schuppers et al., 2010). This paper seeks to extend this methodology to examine the effect of alternative testing strategies and bio-exclusion practices in test-negative herds on probability of JD freedom over time. The work has been developed in an Irish context, where a national JD control program is currently being designed (More et al., 2011).

MATERIALS AND METHODS

The Model

A stochastic simulation model was developed in the R Programming environment (R v 2.12.1; R Project for Statistical Computing, Vienna, Austria), with a web-based interface for ease of user input. The model simulates testing of cattle herds for the presence of Map infection and allows the evaluation and comparison of a variety of testing strategies and alternative tests. Key model inputs are entered as probability distributions to reflect uncertainty about their true values, and the model was run for 1,000 iterations for all simulations, so that outputs are also presented as probability distributions. The model web interface is available at http://epitools.ausvet.com.au/content.php?page=AHI_JD.

The model has 2 primary outputs. The first is an estimate of herd sensitivity (***SeH***) of a given testing strategy; that is, the probability of detecting infection in a herd if it is present at a true prevalence exceeding a specified threshold value or design prevalence. The second is the level of confidence that the true prevalence is less than the specified design prevalence value, assuming negative testing results (***ProbF***), equivalent to the negative predictive value of the herd test; *ProbF* can be estimated over multiple time periods assuming periodic (e.g., annual) testing and allowing for the probability of introduction during the intervening period, based on the numbers of animals introduced and the likelihood of infection in introduced animals. If a positive result on a definitive test such as fecal culture is recorded at any time, the herd is considered infected and calculation of *SeH* and *ProbF* is unnecessary.

Modeling was conducted using a range of small to moderate herd sizes, based on Irish data (Supplemental Table S1; http://www.journalofdairyscience.org/). As a consequence, *SeH* was estimated using an approximation of the hypergeometric probability distribution, as follows:

$$SeH = 1 - (1 - \mathrm{Se} \times n/N)^{d},$$

assuming individual animal specificity (**Sp**) = 100% (after follow-up testing with a highly specific, confirmatory test such as fecal culture), and where Se = individual animal sensitivity, n = sample size, N = population size (MacDiarmid, 1988), and d (the assumed number of infected animals in the herd) = P* × N rounded up to next integer, where P* is the specified design prevalence. For testing strategies in which multiple tests were used and interpreted in series (e.g., serum ELISA followed by fecal culture), $\mathrm{Se} = \mathrm{Se}_1 \times \mathrm{Se}_2 \ldots \times \mathrm{Se}_n$. For testing options in which Sp < 100% (serum or milk ELISA without follow-up), *SeH* was estimated using the modified hypergeometric probability distribution, as described by Cameron and Baldock (1998).

Confidence that the true prevalence is less than P* was estimated as follows:

$$ProbF = \mathrm{NPV} = [1 - (1 - PriorF)]/[1 - (1 - PriorF) \times SeH],$$

where NPV = negative predictive value and *PriorF* is the level of confidence that the prevalence is less than P* before the testing was undertaken (Martin et al.,