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Evaluation of testing strategies to identify infected animals at a single round of testing within dairy herds known to be infected with *Mycobacterium avium* ssp. *paratuberculosis*

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ABSTRACT

As part of a broader control strategy within herds known to be infected with *Mycobacterium avium* ssp. *paratuberculosis* (MAP), individual animal testing is generally conducted to identify infected animals for action, usually culling. Opportunities are now available to quantitatively compare different testing strategies (combinations of tests) in known infected herds. This study evaluates the effectiveness, cost, and cost-effectiveness of different testing strategies to identify infected animals at a single round of testing within dairy herds known to be MAP infected. A model was developed, taking account of both within-herd infection dynamics and test performance, to simulate the use of different tests at a single round of testing in a known infected herd. Model inputs included the number of animals at different stages of infection, the sensitivity and specificity of each test, and the costs of testing and culling. Testing strategies included either milk or serum ELISA alone or with fecal culture in series. Model outputs included effectiveness (detection fraction, the proportion of truly infected animals in the herd that are successfully detected by the testing strategy), cost, and cost-effectiveness (testing cost per true positive detected, total cost per true positive detected). Several assumptions were made: MAP was introduced with a single animal and no management interventions were implemented to limit within-herd transmission of MAP before this test. In medium herds, between 7 and 26% of infected animals are detected at a single round of testing, the former using the milk ELISA and fecal culture in series 5 yr after MAP introduction and the latter using fecal culture alone 15 yr after MAP introduction. The combined costs of testing and culling at a single round of testing increases with time since introduction of MAP infection, with culling costs being much greater than testing costs. The cost-effectiveness of testing varied by testing strategy. It was also greater at 5 yr, compared with 10 or 15 yr, since MAP introduction, highlighting the importance of early detection. Future work is needed to evaluate these testing strategies in subsequent rounds of testing as well as accounting for different herd dynamics and different levels of herd biocontainment.

Key words: Johne's disease, testing strategies, infected herd, control, evaluation

INTRODUCTION

In herds known to be infected with *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**), the causative agent of Johne's disease (**JD**), control is undertaken to limit within-herd transmission (biocontainment), to identify MAP-infected animals from the herd (individual animal testing), and to prevent entry of infected animals into the herd (bioexclusion; Garry, 2011). Risk factors for within-herd transmission are increasingly understood (Doré et al., 2012), and clear recommendations are available for both beef and dairy herds to prevent exposure of susceptible animals to MAP (Garry, 2011; Roussel, 2011). A broad range of control options are being applied internationally, with some differences between countries (Geraghty et al., 2014).

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As part of a broader control strategy within known infected herds, individual animal testing is generally conducted to identify infected animals for action, usually culling. Prompt removal of infected animals is critical to effective on-farm control. However, individual animal testing is problematic. Due to the poor sensitivity (**Se**) of currently available tests (Nielsen and Toft, 2008), this testing is unable to identify all infected animals, particularly those at an early stage of infection, thereby risking ongoing within-herd transmission. Consequently, several authors have suggested that test-and-cull strategies are unlikely to be effective in limiting within-herd transmission without associated changes in management (Groenendaal et al., 2002; Kudahl et al., 2007).

In recent years, substantial advances have occurred in knowledge underpinning the development and interpretation of these testing strategies, relating both to test performance and to the epidemiology of infection in infected herds. Test performance is known to vary by age and stage of infection (Nielsen, 2008; Nielsen and Toft, 2008) and by stage of lactation (Nielsen and Ersbøll, 2006; Nielsen and Toft, 2012). Further, detailed work has been conducted using ELISA to identify animals at greatest risk of future MAP shedding (Toft et al., 2005; Nielsen, 2008). Concurrently, epidemiological aspects of MAP infection in infected herds are becoming increasingly understood, as outlined in recent reviews, such as evidence for in utero infection (Whittington and Windsor, 2009) and age susceptibility of cattle to JD (Windsor and Whittington, 2010). Several epidemiological models of within-herd transmission of MAP in cattle have been also created, as reviewed by Marcé et al. (2010), allowing an opportunity to test hypotheses of MAP transmission and to compare different JD control strategies.

With these advances in knowledge of both test performance and within-herd infection dynamics, opportunities are now available to quantitatively compare different testing strategies in known infected herds. Parallels can be drawn to animal health-surveillance programs, where new methodologies are developing to objectively assess surveillance quality, using quantifiable measures such as effectiveness, cost, and cost-effectiveness (Cameron, 2012). In the context of JD, effectiveness can be measured in terms of detection fraction (defined as the proportion of MAP-infected animals in the herd that are detected), cost based on both testing costs and costs associated with positive test results (assumed to be culling of animals), and cost-effectiveness as either the testing cost or total cost per true positive detected. Detection fraction is similar to test sensitivity (the proportion of infected animals that are test positive), but considers the proportion of all infected animals in the herd (whether tested or not) that are test positive, thereby taking account of the overall testing strategy. Knowledge of these factors would assist individual farmers, veterinary practitioners, program managers, and legislators in the application of testing strategies as part of a broader control program in MAP infected herds. Therefore, our study evaluates the effectiveness, cost, and cost-effectiveness of different testing strategies (combinations of tests) to identify infected animals at a single round of testing within dairy herds known to be MAP infected.

MATERIALS AND METHODS

The Model

A model was developed to simulate the use of different tests at a single round of testing in a single herd. The purpose of the model was to compare the possible costs and effects of different testing strategies by estimating: the prevalence of infected animals in different stages of infection; the detection fraction (the proportion of true positive animals successfully detected); the number of false positives; the herd testing and culling costs; and the prevalence of infection in the herd after culling any positive animals detected.

The model took account of both infection dynamics within a single herd (in terms of age class, stage of infection, and herd size) and test performance (including the possible combination of different screening and confirmatory tests in either series or parallel). Within-herd infection dynamics varied by time since infection was first introduced, whereas the Se and specificity (**Sp**) of each test could vary by stage of infection. The model allowed for inclusion or exclusion of different age classes from testing. However, for the purposes of our study, it was assumed that all animals >2 yr of age were tested. The model was run stochastically using PopTools version 3.2.5 (Hood, 2010) and Microsoft Excel (Microsoft Corp., Redmond, WA) to account for uncertainty around Se and Sp estimates and variability in the spread of the infection at different times after introduction.

Model Inputs

Each of the inputs listed below was required in addition to herd and test strategy descriptions (specified by the user).

Number of Animals by Age Class, Stage of Infection, and Herd Size (Outputs from the Marcé Model). Outputs from a published stochastic compartmental model (Marcé et al., 2011a,b) were used to predict the number of animals by age class,

stage of infection, and herd size. These outputs were also influenced by time since introduction of infection. Briefly, the Marcé model coupled herd dynamics and the infection process. In this model, herd demographics and management were considered typical of a western European dairy herd, including all-year calving and the sale of male calves before 4 wk of age. Calves were not housed in the same pen as adults but were indirectly in contact with the contaminated farm environment. Calves were raised in individual pens for 2 wk, then moved into group pens. Grazing occurred from April to November. Closed herds (those with no animal purchases) were modeled to ensure that the predicted prevalence was reflective of within-herd infection processes, and not due to persistence as a result of animal movements. Herd size remained almost constant, after balancing the sale of heifers and cow mortality or culling with female calf recruitment. Susceptibility to infection was limited to animals before 1 yr of age, with susceptibility in this age group decreasing with age. Animals not infected at 1 yr of age were assumed resistant to MAP infection. All known routes of transmission were considered, including indirect calf-to-calf and adult-to-calf MAP transmission, in utero transmission, and transmission through contaminated milk or colostrum. Environmental contamination was explicitly modeled, considering both contamination of the calf housing facilities and of the whole farm. Both adults and calves were potentially infectious, with the quantity of organism shed varying with age and health or disease state. The probability of culling increased for clinically affected animals, which were present for an average of 6 mo before culling. The Marcé model was run in discrete time steps, with a time interval of 1 wk. Infected herds were created by introducing a single infectious but not shedding or showing clinical signs, first-parity cow into a fully susceptible herd, with no further introductions. The model was run in Scilab 5.3 (www.scilab.org).

As outlined by Nielsen and Toft (2008), 3 mutually exclusive stages of infection (**SI**) could be distinguished from the Marcé model including:

- **SI[infected]**: animals that were infected but not yet shedding or showing clinical signs [from Marcé et al. (2011a), this included the health state of latently infected];
- **SI[infectious]**: animals that were infected and shedding, but not showing clinical signs [from Marcé et al. (2011a), this included the health states of transiently (intermittently) infectious and subclinically infected]; and
- **SI[affected]**: animals that were infected, shedding, and showing clinical signs [from Marcé et al. (2011a), this included the health state of clinically affected].

At any point in time, the total number of animals infected with MAP was equal to the sum of SI[infected], SI[infectious], and SI[affected]. All other animals were considered not infected. From Marcé et al. (2011a), this included the health states of susceptible and resistant. Animals were identifiable across 5 age classes, including unweaned calves (<10 wk of age), weaned calves (from weaning to 1 yr of age), young heifers (from 1 yr to first service), reproductive heifers (from first service to first calving), and cows (from parity 1 to ≥5). Two different herd sizes were considered, including small (~45 cows >2 yr of age) and medium (~140 cows >2 yr of age).

In the current study, we used the baseline scenario during parameterization of the Marcé model, as outlined in Table 2 of Marcé et al. (2011b). After the introduction of one infected animal into a naïve herd, fadeout in the Marcé model occurred in approximately 66% of the runs (Marcé et al., 2011a). Therefore, analyses at each period were restricted to those iterations where MAP prevalence was nonzero. Each simulation was run 500 times.

Se and Sp of Each Test. Three tests were used in the model, including serum ELISA, milk ELISA, and fecal culture, these being tests for which published figures were available relating to Se and Sp at each stage of infection. For each test type and testing matrix, Se and Sp values (minimum, most likely, maximum) were selected for relevant stages of infection (SI[infected], SI[infectious], SI[affected]). For SI[infected] cattle, values selected by the authors in a recent study were used without further revision (Table 1; More et al., 2013). For SI[infectious] and SI[affected] cattle, the authors used the recently published review of operating characteristics (Nielsen and Toft, 2008) supplemented by subsequent relevant peer-reviewed publications (van Weering et al., 2007; Köhler et al., 2008; Vidal-Diez et al., 2009), as outlined below. With respect to SI[infectious] animals, Nielsen and Toft (2008) present 24 Se values for serum ELISA, after excluding kits using LAM (a lipo-arabinomannan preparation) or PPA (a commercial protoplasmic antigen) as antigens. These range from 0.24 to 0.94, with a mean value of 0.47, and were used to define the minimum, maximum, and most likely values (Table 1). The same approach was used for milk ELISA, although the number of data points used was much lower (n = 4). The mean Se values for SI[infectious] cattle generated for serum and milk using this approach (0.46 and 0.41, respectively) indicated an Se of milk relative to serum of 0.89. This was similar to the figure of 0.87 reported by van Weering et al. (2007) for SI[infected] cattle. With respect to SI[affected]

Table 1. Sensitivity (Se) and specificity (Sp) estimates (most likely, range) that were used as model inputs, by test and stage of infection

	Stage of infection (SI)		
Test	SI[Infected]	SI[Infectious]	SI[Affected]
Serum ELISA			
Se	0.15 (0.07–0.22)	0.47 (0.24–0.94)	0.71 (0.50–0.87)
Sp	0.985 (0.95–1.0)	0.985 (0.95–1.0)	0.985 (0.95–1.0)
Milk ELISA			
Se	0.131 (0.061–0.191)	0.41 (0.21–0.61)	0.62 (0.42–0.82)
Sp	0.985 (0.95–1.0)	0.985 (0.95–1.0)	0.985 (0.95–1.0)
Fecal culture			
Se	0.23 (0.16–0.30)	0.74 (0.64–0.84)	0.74 (0.64–0.84)
Sp	1.0 (0.996–1.0)	1.0 (0.996–1.0)	1.0 (0.996–1.0)

animals, 3 values were available for serum ELISA, with a mean Se in cattle of 0.71. In the absence of any published Se values for milk ELISA in affected cattle, a relative Se of 0.87 for milk compared with serum ELISA was used to calculate a value. Based on work by Nielsen et al. (2002), the Se of individual fecal culture as an ancillary (confirmatory) test (on cattle with positive ELISA readings) was considered 0.65 (most likely, range 0.6–0.7). Nielsen and Toft (2008) reported only single Se values for fecal culture for SI[infectious] (0.74) and SI[affected] (0.70) cattle. The higher of these 2 values was used for both, with a 10% estimated range.

Specificity values were not considered to be markedly influenced by the stage of infection. Therefore, the Sp values used previously for SI[infected] animals (More et al., 2013) have also been used for SI[infectious] and SI[affected] animals. An Sp of 1.0 (most likely, range: 0.996–1.0) was used for individual fecal culture, with the range being based on the work of Vidal-Diez et al. (2009). This specificity reflects the standard practice of confirming isolates as MAP through use of PCR, in which stringent primers are employed for targets such as IS900 or F57 (Möbius et al., 2008). The Se and Sp values (most likely, range) used for each test, by stage of infection, are presented in Table 1.

Costs. The testing costs are presented in Table 2, updating earlier work (More et al., 2013). We calculated the increased cost of replacement due to premature culling, relative to a noninfected parity 5 cow, taking account of both age of culling and stage of infection for cattle in all stages of infection (including noninfected cattle to take account of possible false-positive results). We assumed the cost of rearing a replacement heifer to be \$1,639, based on recent Irish work (Shalloo et al., 2012), and that all cull cows were slaughtered at parity end, with a salvage value for noninfected cows of \$629 (cold carcass weight of 240 kg, \$2.62/kg paid for cull boner cows in Ireland during June 2013). Therefore, the estimated increased cost of replacement due to premature culling of noninfected first-parity animals was \$1,010 (being \$1,639 − \$629). For older, noninfected animals, this figure was depreciated in a linear manner, to \$0 for parity 5 cows. The salvage value of SI[infected] cows was assumed to be the same as noninfected cows; therefore, the estimated increased costs were equivalent. For SI[infectious] cows, a 12.4% reduction in carcass weight was assumed compared with noninfected and SI[infected] cows, guided by a similar difference between ELISA negative and positive animals in a recent Spanish study (Vázquez et al., 2012). No account was taken of transport costs to the abattoir, as these vary greatly depending on distance traveled, number of animals being transported, and so on. Therefore, the estimated increased cost of replacement due to premature culling of all SI[infectious] cows, in comparison to noninfected cows of equivalent parity, was increased by \$78 (12.4% of \$629). For SI[affected] cows, we assumed that no animals entered the food chain; therefore, any salvage value was foregone. In addition, farmers incur a knackery cost (for transport and disposal, subsidized in Ireland for animals >48 mo) to the farmer of \$52.53 (increased to \$52.83 during rounding to euros; Department of Agriculture, Food and the Marine, 2013). For these reasons, the estimated increased cost of replacement due to premature culling of all SI[affected] cows of equivalent parity, in comparison to both noninfected and SI[infected] cows, was increased by \$681.83 (\$629 + 52.83). All costs are presented in US dollars, assuming an exchange of €1 = \$1.10328.

Model Calculations

The model partitioned the herd by age or parity class and stage of infection. For each age or parity class, a range of measures were calculated and then summarized across the entire herd. These included the proportion of the herd infected with MAP (regardless of the

Table 2. Current indicative costs associated with Johne's disease testing in Ireland

Test	Cost[1] ($)		
	Visit fee (per farm)	Collection fee (per cow/sample)	Laboratory testing fee (per sample)[1]
Serum ELISA			
As part of the national brucellosis surveillance program	—	—	3.86
Otherwise	—	3.96[2]	3.86
Milk ELISA			
As part of routine milk recording	—	—	2.76
Otherwise	—	1.10[2]	2.76
Fecal culture	55.16	3.31	38.06

[1]Based on quotes obtained from various Irish laboratories (October 2013), assuming €1 = $1.10328.

[2]The collection fee incorporates a farm visit fee and is charged per sample collected.

stage of infection) that would be detected, which was calculated as:

$$P_{as} = I_{as} \times S_a \times Se_s,$$

where P_{as} is the proportion of animals in age class a and stage of infection s that were correctly identified as infected with MAP; I_{as} is the proportion of truly infected animals in age class a and stage of infection s that existed in the herd; S_a is the proportion of the age class that were tested; and Se_s is the combined Se of the testing strategy for that stage of infection.

The number of false positives in each age class was calculated as:

$$FP_a = Nu_a \times S_a \times (1 - Sp),$$

where FP_a is the number of false positive animals in the age class; Nu_a is the number of uninfected animals in the age class; S_a is the proportion of animals in that age class that were tested; and Sp is the combined test Sp of the testing strategy used.

The ending prevalence (prevalence after removal of test-positive animals, which includes both true and false positives) was calculated as the number of remaining infected animals (either not tested or false negatives) divided by the herd size after removal of test-positive animals. All test-positive animals were assumed to have been culled.

Scenarios Considered

In total, 54 different scenarios were evaluated, relating to 2 different dairy herd sizes (small: ~45 cows, >2 yr of age; medium: ~140 cows, >2 yr of age) and 3 different periods since introduction of MAP infection (for simplicity, 5, 10, and 15 yr). These scenarios were also based on 5 different testing strategies (increasing to 9 after accounting for different costing structures for serum and milk ELISA): serum ELISA alone (with and without a collection fee per sample, the latter applicable to serum collection as part of the national brucellosis surveillance program); serum ELISA (again with and without a collection fee) followed by fecal culture if ELISA-positive; milk ELISA alone (with and without a collection fee per sample, the latter applicable on farms with routine milk recording); milk ELISA (with and without a collection fee) followed by fecal culture if ELISA-positive; and fecal culture alone.

Model Outputs

The model outputs include 70 different measures of herd dynamics, disease, test performance, and cost. This paper presents key measures providing insights into effectiveness, measured in terms of the detection fraction, or the proportion of truly infected animals in the herd that are successfully detected by the testing strategy; true prevalence; apparent prevalence; true positives (an infected animal that tests positive) detected; false positives (a noninfected animal that tests positive) detected; cost, based on test costs and costs associated with positive test results (assumed to be culling of animals); and cost-effectiveness, measured as the cost of testing associated with each true positive detected or the total cost per true positive detected.

Sensitivity Analyses

Sensitivity analyses were conducted to evaluate the effect of variations in model inputs on model outputs. These included 4 analyses. (1) A global decrease in estimated test sensitivity. For each of the Se estimates in Table 1, the revised most likely value was reduced to equidistant between the most likely and minimum values, whereas the range was unchanged. For example, the Se of serum ELISA for SI[Infectious] animals was reduced from 0.47 (0.24–0.94) to 0.335 (0.24–0.94)

[noting that 0.335 = 0.47 – 0.5 × (0.47 – 0.24); Supplementary Table S1; http://dx.doi.org/10.3168/jds.2014-8211]. (2) A global increase in estimated test sensitivity. For each of the Se estimates in Table 1, the revised most likely value was increased to equidistant between the most likely and maximum value, whereas the range was unchanged. For example, the Se of milk ELISA for SI[Affected] animals was increased from 0.62 (0.42–0.82) to 0.72 (0.42–0.82) [noting that 0.72 = 0.62 + 0.5 × (0.82 – 0.62); Supplementary Table S1]. (3) A global decrease in estimated test specificity. For each of the Sp estimates in Table 1, the revised most likely value was decreased to equidistant between the most likely and minimum value, whereas the range was unchanged. For example, the Sp of fecal culture was decreased from 1.0 (0.996–1.0) to 0.998 (0.996 – 1.0) [noting that 0.998 = 1.0 – 50% × (1.0 – 0.996); Supplementary Table S1]. (4) An increase in the estimated costs associated with premature culling, by 10%.

RESULTS

Within-Herd Transmission

The estimated median number of animals at different stages of infection, by herd size, age, and years since introduction of MAP infection, is presented in Table 3. The number of SI[affected], SI[infectious], and SI[infected] animals increased with time since introduction, including several infectious and infected animals that were ≤2 yr of age. The true prevalence and number of animals at different stages of infection in a medium herd, by years since introduction of MAP infection, is presented in Figures 1a and 1b. In a herd with a total of 278 animals (5th and 95th percentile: 261, 296), at 10 yr after MAP introduction and in the absence of on-farm MAP control, there were an estimated 117 (6, 232) infected animals, including 64 (1, 114) SI[infected], 41 (1, 94) SI[infectious], and 4 (0, 14) SI[affected] animals.

Testing Effectiveness

The apparent prevalence of MAP infection in a medium herd at a single round of testing using the serum ELISA and fecal culture in series, by year since introduction, is presented in Figure 2. At 10 yr after MAP introduction, the median true and apparent prevalence was 0.42 (0.02, 0.82) and 0.07 (0.003, 0.22), respectively. Figure 3 presents the proportion of tested cattle in a median herd that are true positives, false positives, false negatives, and true negatives based on a single round of testing using serum ELISA and fecal culture in series on animals >2 yr of age by year of MAP introduction. With increasing time since introduction, an increasing proportion of the tested herd was false negative, rising from 0.03 (0, 0.15) at 5 yr, to 0.51 (0.05, 0.72) at 15 yr after MAP introduction. In contrast, the proportion of false positives was extremely low, consistently less than 2×10^{-6}.

Median estimates of detection fraction (effectiveness) at a single round of testing, by herd size, testing strategy, and at varying times after introduction of MAP infection are presented in Table 4. Detection fraction was low in all testing strategies, being lowest in medium herds when the milk ELISA and fecal culture were used in series [0.07 (0, 0.24), 5 yr after introduction of infection] and highest when fecal culture was used alone [0.26 (0.12, 0.53), 15 yr after introduction of infection]. For a given testing strategy, relative little difference was noted in the median estimate of detection fraction by either herd size or time since MAP introduction.

Testing and Culling Costs

The median estimates of costs of testing and culling at a single round of testing in an infected herd, by herd size, testing strategy, and time since introduction of MAP infection, are presented in Table 5. In these calculations it was assumed that all test-positive animals were culled. At each time following MAP introduction, the use of milk ELISA and fecal culture was associated with the lowest cost of testing and culling, followed by serum ELISA and fecal culture, milk ELISA alone, serum ELISA alone, then fecal culture. Culling costs were substantially greater than testing costs, with the different pricing structures for serum and milk ELISA (e.g., as part of the national brucellosis surveillance program, otherwise; see Table 2) having little effect on the total cost (incorporating both testing and culling costs). Culling costs increased substantially with increasing time since MAP introduction.

Cost per True Positive Detected

The median estimates of the testing and the total cost per true positive detected at a single round of testing in an infected dairy herd, by herd size, testing strategy, and time since introduction of MAP infection, are presented in Tables 6 and 7, respectively. At 10 yr since MAP introduction, the estimates of testing cost per true positive detected varied considerably across testing strategy, varying between $34 (11, 839; milk

Table 3. Estimated median (5th percentile, 95th percentile) number of animals at different stages of infection (SI), by herd size, age, and years since introduction of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection[1]

Item	Years since introduction of MAP infection		
	5	10	15
Small herds (~45 cows greater than 2 yr of age)			
All cattle	88 (78, 98)	87 (76, 98)	87 (70, 98)
Cattle ≤2 yr	43 (33, 54)	42 (33, 54)	44 (34, 62)
Cattle >2 yr	45 (41, 50)	45 (39, 49)	43 (31, 49)
No. of uninfected animals	75 (50, 94)	50 (21, 88)	27 (7, 93)
Cattle ≤2 yr	35 (15, 48)	20 (5, 42)	10 (0, 36)
Cattle >2 yr	41 (29, 47)	31 (12, 47)	15 (4, 45)
No. of infected animals, by SI			
All cattle			
Total infected	12 (1, 38)	35 (2, 67)	62 (13, 93)
SI[Infected]	7 (0, 28)	21 (1, 35)	31 (0, 46)
SI[Infectious]	4 (0, 16)	12 (1, 29)	23 (0, 36)
SI[Affected]	0 (0, 2)	1 (0, 5)	3 (0, 9)
Cattle ≤2 yr of age			
SI[Infected]	5 (0, 23)	15 (0, 27)	22 (0, 33)
SI[Infectious]	1 (0, 12)	5 (0, 16)	7 (0, 18)
SI[Affected]	0 (0, 0)	0 (0, 0)	0 (0, 0)
Cattle >2 yr of age			
SI[Infected]	1 (0, 7)	5 (0, 13)	10 (1, 17)
SI[Infectious]	2 (0, 7)	7 (0, 16)	15 (2, 26)
SI[Affected]	0 (0, 2)	1 (0, 5)	3 (0, 14)
Medium herds (~140 cows greater than 2 yr of age)			
All cattle	277 (256, 298)	278 (261, 296)	278 (255, 296)
Cattle ≤2 yr	135 (113, 154)	137 (121, 157)	140 (123, 160)
Cattle >2 yr	142 (135, 149)	140 (133, 149)	140 (125, 147)
No. of uninfected animals	258 (171, 281)	167 (68, 281)	65 (20, 280)
Cattle ≤2 yr	120 (59, 145)	64 (18, 134)	22 (2, 113)
Cattle >2 yr	136 (113, 145)	104 (43, 144)	43 (9, 142)
No. of infected animals, by SI			
All cattle			
Total infected	18 (2, 99)	117 (6, 232)	214 (28, 274)
SI[Infected]	10 (0, 66)	64 (1, 114)	112 (2, 141)
SI[Infectious]	6 (1, 38)	41 (1, 94)	83 (3, 120)
SI[Affected]	1 (0, 4)	4 (0, 14)	12 (0, 23)
Cattle ≤2 yr of age			
SI[Infected]	5 (0, 23)	15 (0, 27)	22 (0, 33)
SI[Infectious]	1 (0, 12)	5 (0, 16)	7 (0, 18)
SI[Affected]	0 (0, 0)	0 (0, 0)	0 (0, 0)
Cattle >2 yr of age			
SI[Infected]	2 (0, 12)	16 (0, 39)	33 (4, 52)
SI[Infectious]	2 (0, 13)	18 (1, 55)	51 (4, 77)
SI[Affected]	1 (0, 4)	4 (0, 15)	12 (1, 26)

[1]Inconsistencies between the totals and the sum of their constituents are due to stochasticity and rounding during modeling. Measures of variability, applicable to each estimate, are not presented here.

ELISA alone as part of routine milk recording) and $300 (103, 8,028; fecal culture alone) in medium herds (Table 6). The estimates were substantially higher at 5 yr since introduction. The estimates of total cost per true positive detected were very similar across both herd size and testing strategy, varying between $844 (688, 6,194; serum ELISA alone as part of the national brucellosis surveillance program) and $1,008 (810, 9,357; fecal culture alone) in small herds and between $865 (729, 5,632; serum ELISA alone as part of the national brucellosis surveillance program) and $1,061 (826, 9,059; fecal culture alone) in medium herds 10 yr since MAP introduction (Table 7). The estimates were also higher at 5 yr since introduction.

Sensitivity Analysis

There was some effect of changes to model inputs on the median estimates of testing cost per true positive detected at a single round of testing in an infected dairy herd (Supplementary Table S2; http://dx.doi.org/10.3168/jds.2014-8211); testing costs per true positive detected decreased with increased test sensitivity, increased with decreased test sensitivity, but changed little with decreased test specificity. The effect of these changes, and of increased culling costs, on the median estimates of total cost per true positive detected was limited (Supplementary Table S3; http://dx.doi.org/10.3168/jds.2014-8211).

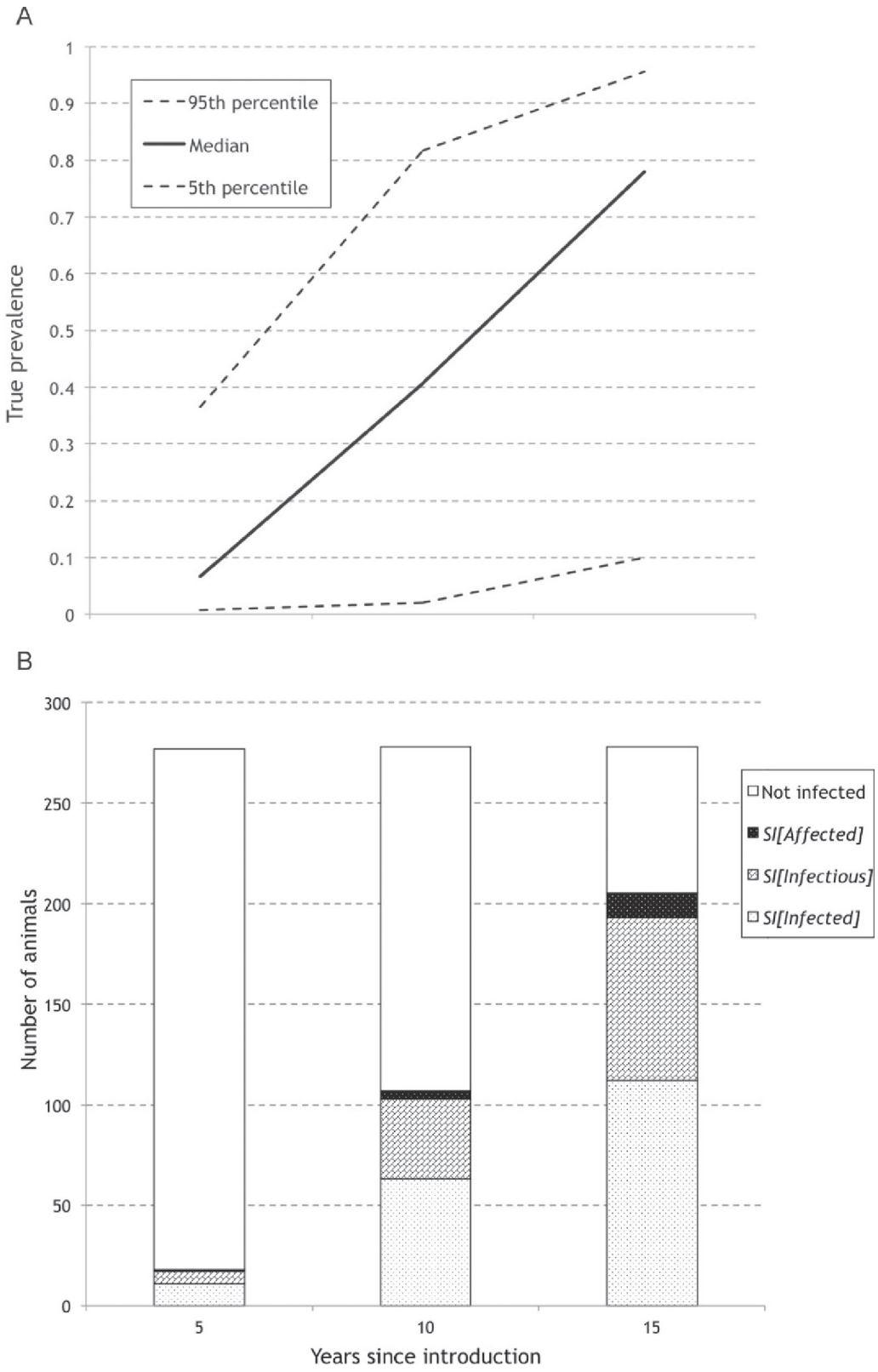

Figure 1. Estimates of (a) the true prevalence and (b) the median number of animals at different stages of infection (SI) in a medium dairy herd of ~140 cows >2 yr of age at 5, 10, and 15 yr following the introduction of *Mycobacterium avium* ssp. *paratuberculosis* infection. After the introduction of 1 SI[infectious] animal into a naïve herd, fadeout occurred in approximately 66% of the runs. In the current study, analyses at each time period were restricted to those iterations where *Mycobacterium avium* ssp. *paratuberculosis* prevalence was nonzero.

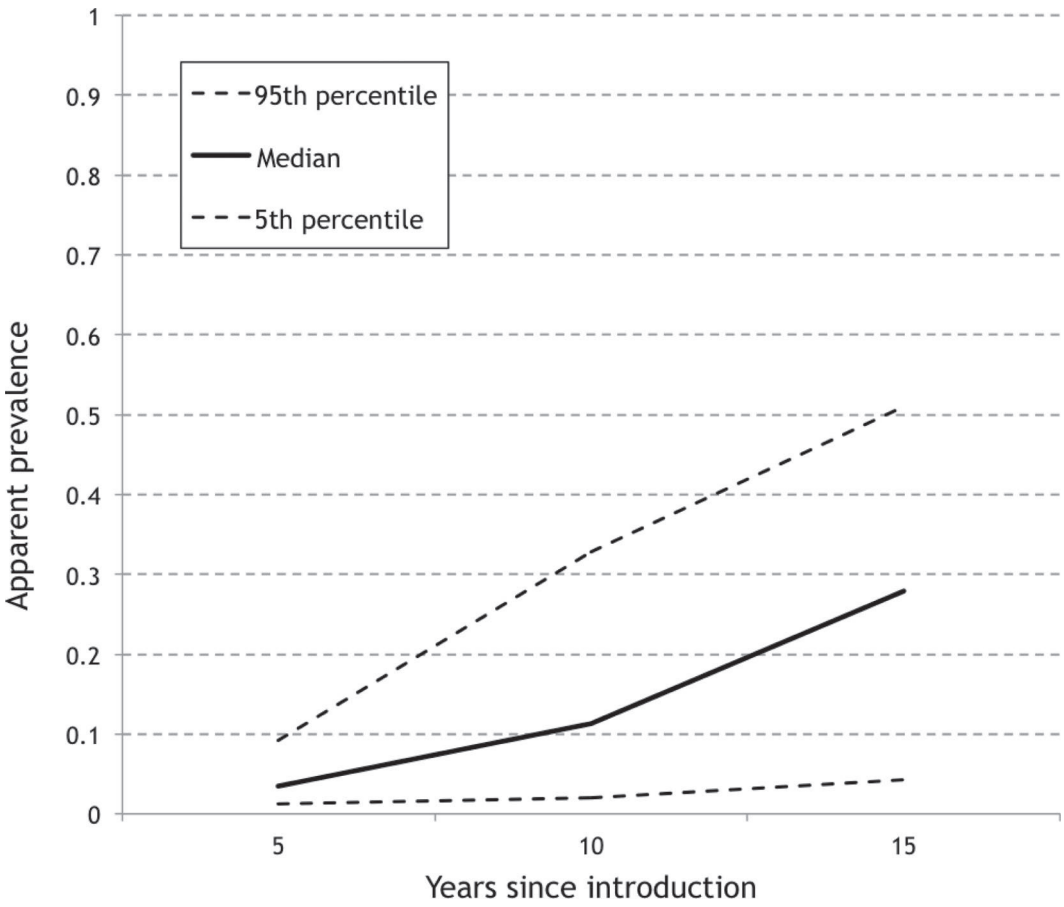

Figure 2. Apparent prevalence of *Mycobacterium avium* ssp. *paratuberculosis* infection in a medium dairy herd of ~140 cows greater than 2 yr of age at a single round of testing using the serum ELISA and fecal culture in series, by year since introduction.

DISCUSSION

In infected herds in Ireland, as elsewhere, herd owners and their veterinarians are seeking to implement efficient control measures and then use annual or semi-annual testing to identify infected cattle for segregation and culling, and to do this in a cost-effective manner. A national pilot JD-control program was recently introduced to assist herd owners and their private veterinarians in their efforts toward JD control and prevention, both in noninfected and infected herds. The current study sought to provide robust evidence to support the use of cost-effective testing strategies in infected herds, informed by test methods, herd sizes, and costs that are relevant to the Irish industry. The current study is also informed by available knowledge about within-herd prevalence (low in many infected herds; Good et al., 2009) and time since herds were first infected (often relatively recently, within the last 15 yr, based on work by Barrett et al., 2011). The key findings should be of wider relevance and interest.

In both small and medium herds, detection fraction was quite low across all testing strategies. Detection fraction was highest when fecal culture was used alone, reflecting the greater Se of this method, particularly among SI[infected] animals (Table 1). Any increase in test Se, while holding test Sp constant, would result in an increase in a detection fraction. This would be particularly marked if test Se were increased for SI[infected] and SI[infectious] animals, as these are the most frequent stages of infection in infected herds (Table 3). In addition, detection fraction was consistently greater when using an ELISA alone (either serum or milk) than with fecal culture in series (Table 4). This effect is a reflection of the increased Sp, but decreased Se, that is achieved when testing in series, but also the greater relative frequency of false negatives to false positive test results with both testing strategies (Figure 3). False-negative results are of major importance with increasing time since MAP introduction and regardless of the testing strategy, whereas the probability of false-positive results is negligible (Figure 3). There are

Table 4. Median (5th percentile, 95th percentile) estimates of effectiveness of a single round of testing in an infected dairy herd by herd size, testing strategy, and time since introduction of infection[1]

	Years since introduction of *Mycobacterium avium* ssp. *paratuberculosis* infection		
Herd size and testing strategy	5	10	15
Small herds (~45 cows >2 yr of age)			
Serum ELISA			
Alone	0.14 (0, 0.43)	0.16 (0.03, 0.36)	0.19 (0.09, 0.57)
With fecal culture in series	0.09 (0, 0.31)	0.11 (0.01, 0.25)	0.13 (0.06, 0.34)
Milk ELISA			
Alone	0.12 (0, 0.36)	0.13 (0.03, 0.28)	0.16 (0.08, 0.33)
With fecal culture in series	0.07 (0, 0.25)	0.09 (0.01, 0.20)	0.11 (0.05, 0.22)
Fecal culture			
Alone	0.21 (0, 0.62)	0.23 (0.05, 0.46)	0.26 (0.13, 0.52)
Medium herds (~140 cows >2 yr of age)			
Serum ELISA			
Alone	0.13 (0.01, 0.41)	0.15 (0.04, 0.34)	0.18 (0.09, 0.42)
With fecal culture in series	0.09 (0, 0.29)	0.10 (0.03, 0.23)	0.12 (0.05, 0.27)
Milk ELISA			
Alone	0.11 (0, 0.35)	0.12 (0.04, 0.28)	0.15 (0.07, 0.29)
With fecal culture in series	0.07 (0, 0.24)	0.08 (0.03, 0.19)	0.10 (0.04, 0.24)
Fecal culture			
Alone	0.17 (0, 0.54)	0.21 (0.07. 0.44)	0.26 (0.12, 0.53)

[1]Effectiveness is measured using detection fraction, defined as the number of true positives divided by the total number of infected animals.

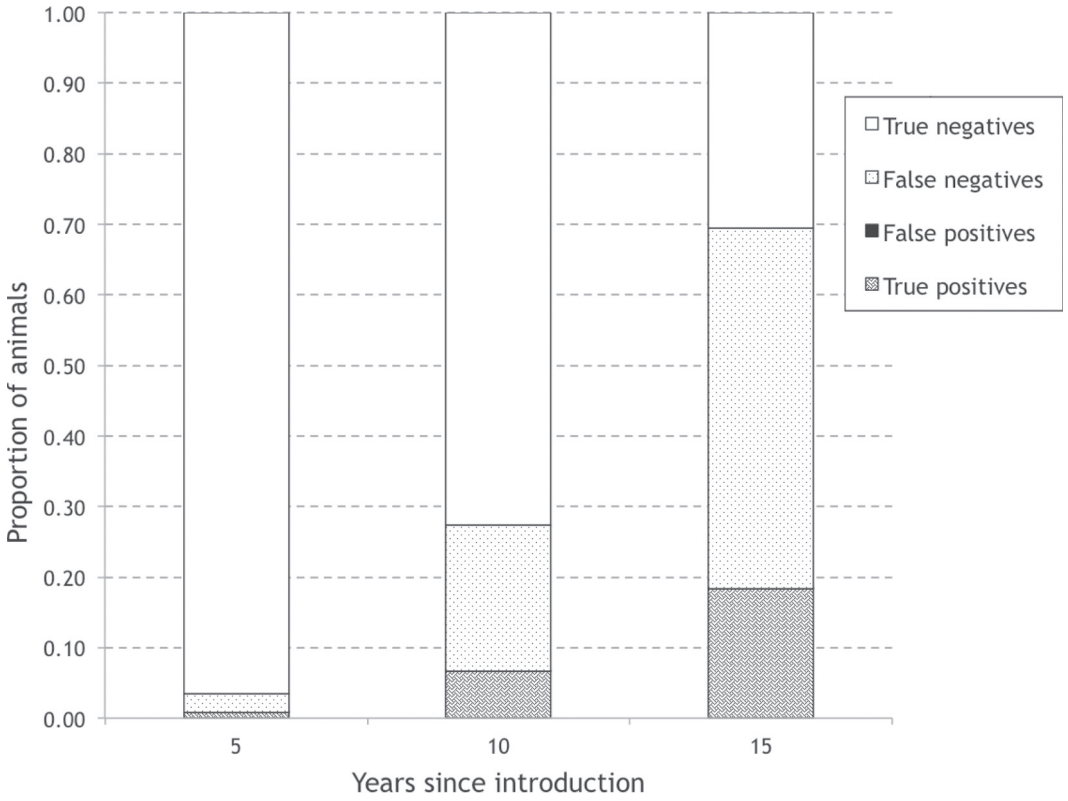

Figure 3. The median proportion of all tested cattle in a medium dairy herd that are true positives, false positives, false negative, and true negatives, based on a single round of testing using serum ELISA and fecal culture in series on animals >2 yr of age, at 5, 10, and 15 yr since *Mycobacterium avium* ssp. *paratuberculosis* introduction. At each time period, the proportion of false positives (the number of positives results in noninfected animals, divided by the herd size) was very low ($<2 \times 10^{-6}$).

Table 5. Median (5th percentile, 95th percentile) estimates of cost (in $[1]) of testing and culling at a single round of testing in an infected dairy herd, by herd size, testing strategy, and time since introduction of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection (it is assumed that all test-positive animals were culled)

	Years since introduction of MAP infection		
Herd size and testing strategy	5	10	15
Small herds (~45 cows >2 yr of age)			
Testing costs			
Serum ELISA			
As part of the national brucellosis surveillance program			
Alone	174 (158, 193)	174 (151, 189)	170 (143, 209)
With fecal culture in series	293 (229, 472)	456 (241, 793)	702 (304, 1,174)
Otherwise			
Alone	352 (321, 391)	352 (305, 383)	344 (289, 422)
With fecal culture in series	471 (405, 650)	630 (415, 968)	861 (484, 1,339)
Milk ELISA			
As part of routine milk recording			
Alone	124 (113, 138)	124 (108, 135)	121 (105, 141)
With fecal culture in series	232 (181, 383)	368 (188, 655)	559 (216, 960)
Otherwise			
Alone	174 (158, 193)	174 (151, 189)	170 (147, 209)
With fecal culture in series	284 (229, 438)	410 (236, 689)	608 (286, 1,013)
Fecal culture			
Alone	1,917 (1,751, 2,124)	1,917 (1,669, 2,082)	1,876 (1,627, 2,289)
Culling costs			
Serum ELISA			
Alone	1,501 (396, 4,662)	4,181 (539, 9,986)	7,996 (1,401, 17,477)
With fecal culture in series	675 (1, 2,822)	2,647 (21, 6,858)	5,413 (649, 11,635)
Milk ELISA			
Alone	1,351 (366, 3,903)	3,578 (531, 8,367)	6,630 (1,321, 13,478)
With fecal culture in series	568 (1, 2,398)	2,151 (20, 5,784)	4,306 (374, 9,207)
Fecal culture			
Alone	1,541 (40, 5,970)	5,540 (127, 12,988)	11,366 (1,128, 19,032)
Total costs (testing and culling)			
Serum ELISA			
As part of the national brucellosis surveillance program			
Alone	1,677 (569, 4,836)	4,352 (713, 10,156)	8,162 (1,574, 17,655)
With fecal culture in series	974 (237, 3,306)	3,113 (262, 7,627)	6,127 (966, 12,768)
Otherwise			
Alone	1,888 (757, 4,991)	4,727 (897, 10,508)	8,482 (1,811, 19,969)
With fecal culture in series	1,178 (423, 3,514)	3,211 (440, 7,816)	6,103 (1,116, 12,496)
Milk ELISA			
As part of routine milk recording			
Alone	1,475 (489, 4,023)	3,701 (661, 8,483)	6,751 (1,446, 13,605)
With fecal culture in series	805 (188, 2,768)	2,522 (211, 6,421)	4,865 (579, 10,183)
Otherwise			
Alone	1,511 (541, 4,015)	3,779 (705, 8,651)	6,812 (1,322, 14,350)
With fecal culture in series	879 (237, 2,831)	2,487 (257, 6,110)	4,954 (747, 10,432)
Fecal culture			
Alone	3,463 (1,965, 7,881)	7,400 (2,092, 14,860)	13,161 (3,087, 21,145)
Medium herds (~140 cows >2 yr of age)			
Testing costs			
Serum ELISA			
As part of the national brucellosis surveillance program			
Alone	548 (521, 575)	544 (514, 583)	541 (490, 587)
With fecal culture in series	692 (610, 1,062)	1,209 (639, 2,445)	2,177 (763, 3,389)
Otherwise			
Alone	1,111 (1,056, 1,166)	1,103 (1,040, 1,173)	1,095 (1,001, 1,189)
With fecal culture in series	1,256 (1,158, 1,616)	1,771 (1,213, 3,047)	2,734 (1,302, 3,903)
Milk ELISA			
As part of routine milk recording			
Alone	392 (372, 411)	389 (367, 414)	386 (350, 416)
With fecal culture in series	519 (452, 825)	950 (473, 1,974)	1,775 (561, 2,819)
Otherwise			
Alone	548 (521, 575)	544 (514, 575)	541 (490, 583)
With fecal culture in series	680 (608, 982)	1,115 (632, 2,201)	1,916 (727, 2,995)
Fecal culture			
Alone	5,930 (5,641, 6,220)	5,889 (5,558, 6,261)	5,847 (5,310, 6,344)

Continued

Table 5 (Continued). Median (5th percentile, 95th percentile) estimates of cost (in $[1]) of testing and culling at a single round of testing in an infected dairy herd, by herd size, testing strategy, and time since introduction of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection (it is assumed that all test-positive animals were culled)

	Years since introduction of MAP infection		
Herd size and testing strategy	5	10	15
Culling costs			
Serum ELISA			
Alone	3,073 (1,061, 9,136)	11,308 (1,692, 33,574)	27,501 (3,951, 49,682)
With fecal culture in series	905 (4, 5,543)	6,967 (246, 21,967)	18,042 (1,490, 32,991)
Milk ELISA			
Alone	2,825 (967, 8,013)	9,820 (1,719, 27,874)	22,619 (3,264, 39,823)
With fecal culture in series	743 (4, 4,393)	5,687 (175, 18,206)	15,186 (1,041, 28,439)
Fecal culture			
Alone	1,944 (103, 11,752)	14,871 (541, 45,075)	39,135 (3,201, 56,733)
Total costs (testing and culling)			
Serum ELISA			
As part of the national brucellosis surveillance program			
Alone	2,628 (1,611, 9,666)	11,862 (2,253, 34,127)	28,039 (4,505, 50,215)
With fecal culture in series	1,594 (628, 6,601)	8,161 (874, 24,354)	20,224 (2,233, 36,413)
Otherwise			
Alone	4,156 (2,140, 10,556)	12,676 (2,883, 34,174)	28,584 (4,694, 50,532)
With fecal culture in series	2,129 (1,191, 7,066)	8,800 (1,460, 25,774)	20,868 (2,554, 36,557)
Milk ELISA			
As part of routine milk recording			
Alone	3,219 (1,359, 8,404)	10,213 (2,110, 28,279)	23,008 (3,666, 40,226)
With fecal culture in series	1,259 (467, 5,203)	6,641 (644, 20,182)	16,959 (1,604, 31,155)
Otherwise			
Alone	3,435 (1,508, 8,324)	10,275 (2,313, 28,217)	23,233 (3,784, 40,237)
With fecal culture in series	1,453 (626, 5,492)	6,894 (823, 21,214)	16,908 (1,860, 31,520)
Fecal culture			
Alone	7,924 (6,100, 17,664)	20,765 (6,531, 50,888)	44,939 (9,288, 62,664)

[1]Assuming €1 = $1.10328.

several reasons for the observed increase in detection fraction with time since MAP introduction (Table 4). There was an increase in the percentage of infected animals that were >2 yr of age, and therefore tested (in medium herds: 28 and 45% at 5 and 15 yr, respectively; derived from Table 3), effectively leading to an increase in testing coverage. Further, among the tested population (animals >2 yr of age), those at the SI[infected] stage of infection, which is associated with the poorest test Se, are a decreasing percentage of all infected animals (in medium herds: 40 and 34% at 5 and 15 yr, respectively; again derived from Table 3) with increasing time since MAP introduction. Therefore, the increase in detection fraction is associated with increases in both testing coverage (the proportion of infected animals under test) and overall Se of each testing strategy.

The combined costs of testing and culling at a single round of testing increases with time since introduction of MAP infection, with culling costs being much greater than testing costs (Table 5). For example, in a medium dairy herd where MAP had been introduced 15 yr previously, the estimated costs of testing and culling, using the milk ELISA and fecal culture in series, were $1,775 (5th and 95th percentiles: 561, 2,819; as part of milk recording) and $15,186 (1,041, 28,439), respectively. However, these results need to be interpreted with caution for several reasons. In our study, the increased cost of replacement due to premature culling was calculated relative to a noninfected parity 5 cow after accounting for both age of culling and stage of infection for cattle in all stages of infection. We accept that this will result in an overestimate of the true cost of premature culling, noting that infected cows on average will not survive as long as uninfected cows (Raizman et al., 2007). This issue will be resolved once accurate estimates become available on the average reduction in longevity of SI[infected], SI[infectious], and SI[affected] animals. Some quantitative information about survival of infected cows is available (e.g., Raizman et al., 2007), but not directly applicable to these stages of infection. In addition, the results are subject to considerable uncertainty, as reflected in the wide confidence limits. It is also assumed that all test-positive animals are culled, with culling costs taking account of each animal's stage of infection and salvageable value for each culled animal. In practice, it is acknowledged that culling of large numbers of test-positive animals at a single point in time is not feasible, because farmers are only able to cull a defined number of infected animals without adversely affecting general farm management. Nonetheless, these increasing cull costs reflect both

Table 6. Median (5th percentile, 95th percentile) estimates of the testing cost per true positive detected (in $[1]) at a single round of testing in an infected dairy herd, by herd size, testing strategy, and time since introduction of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection

	Years since introduction of MAP infection		
Herd size and testing strategy	5	10	15
Small herds (~45 cows >2 yr of age)			
Serum ELISA			
As part of the national brucellosis surveillance program			
Alone	108 (30, 738)	32 (13, 409)	15 (9, 97)
With fecal culture in series	286 (122, 4,258)	127 (84, 883)	92 (76, 271)
Otherwise			
Alone	215 (60, 1,332)	63 (26, 831)	30 (18, 182)
With fecal culture in series	452 (169, 7,775)	177 (103, 1,645)	115 (89, 410)
Milk ELISA			
As part of routine milk recording			
Alone	93 (25, 552)	27 (11, 383)	13 (8, 75)
With fecal culture in series	276 (120, 4,044)	124 (83, 879)	92 (75, 259)
Otherwise			
Alone	131 (36, 939)	38 (15, 500)	18 (11, 110)
With fecal culture in series	332 (134, 4,363)	144 (91, 1,209)	100 (79, 301)
Fecal culture			
Alone	812 (238, 5,384)	250 (107, 2,835)	115 (81, 740)
Medium herds (~140 cows >2 yr of age)			
Serum ELISA			
As part of the national brucellosis surveillance program			
Alone	267 (50, 1,280)	40 (13, 1,045)	14 (9, 178)
With fecal culture in series	513 (143, 2,273)	131 (82, 1,587)	87 (73, 355)
Otherwise			
Alone	519 (102, 2,604)	79 (27, 2,020)	29 (18, 391)
With fecal culture in series	931 (222, 4,435)	191 (102, 2,709)	109 (86, 726)
Milk ELISA			
As part of routine milk recording			
Alone	224 (42, 1,191)	34 (11, 839)	12 (7, 164)
With fecal culture in series	462 (139, 2,396)	126 (80, 1,466)	85 (71, 376)
Otherwise			
Alone	312 (61, 1,605)	48 (15, 1,207)	17 (10, 228)
With fecal culture in series	581 (164, 3,286)	146 (86, 1,828)	92 (76, 440)
Fecal culture			
Alone	1,907 (382, 8,584)	300 (103, 8,028)	108 (77, 1,448)

[1]Assuming €1 = $1.10328.

the considerable cost differences in culling animals in different stages of infection (SI[infected], SI[infectious], and SI[affected]), noting the lack of salvage value for SI[affected] animals and the overall number of animals potentially culled. This underlines the importance of early identification of infected animals, before they progress to SI[affected], and changes to herd management (Dorshorst et al., 2006) over the long-term (Cho et al., 2013) to limit ongoing transmission (and further infected animals). Caution is required when comparing culling costs associated with each testing strategy. As highlighted in Table 5, milk ELISA testing, either alone or in series with fecal culture, has a lower culling cost relative to the comparable serum ELISA cost. These lower costs are ultimately a reflection of the smaller number of truly infected animals detected by the milk ELISA due to its lower Se. Lower culling costs at a given point need to be considered in the context of the reciprocal increased future losses due to JD arising from retention of a greater number of infected animals in the herd. Analysis of the interaction between these costs and benefits is beyond the scope of the current study.

Cost-effectiveness was estimated using 2 measures: testing cost per true positive detected and total cost per true positive detected, each at a single round of testing in a known infected herd. Testing cost per true positive detected varied greatly across testing strategy and by years since introduction of MAP infection. For example, at 10 yr after MAP introduction in a small dairy herd, as a median estimate, it cost $27 (5th and 95th percentiles: 11, 383) per true positive detected when using milk ELISA alone as part of routine milk recording and $124 (83, 879) when this test was used in series with fecal culture. These differences were expected, noting that testing cost per true positive detected is derived from testing costs (which vary greatly by testing strategy; Table 5) and detection fraction (which does not; Table 4). Prevalence increases with time since introduction (Figure 1a), being lowest at 5

Table 7. Median (5th percentile, 95th percentile) estimates of the total cost per true positive detected (in $[1]) at a single round of testing in an infected dairy herd, by herd size, testing strategy, and time since introduction of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) infection

	Years since introduction of MAP infection		
Herd size and testing strategy	5	10	15
Small herds (~45 cows >2 yr of age)			
Serum ELISA			
As part of the national brucellosis surveillance program			
Alone	1,147 (738, 12,435)	844 (688, 6,194)	745 (633, 5,546)
With fecal culture in series	1,069 (710, 12,811)	889 (713, 8,827)	805 (686, 7,646)
Otherwise			
Alone	1,258 (796, 15,098)	872 (712, 6,182)	762 (647, 5,574)
With fecal culture in series	1,231 (819, 24,496)	943 (770, 15,122)	831 (706, 14,942)
Milk ELISA			
As part of routine milk recording			
Alone	1,179 (740, 11,666)	847 (687, 5,891)	747 (633, 6,197)
With fecal culture in series	1,058 (714, 13,582)	888 (706, 7,584)	805 (682, 7,393)
Otherwise			
Alone	1,213 (755, 14,836)	858 (698, 5,904)	754 (642, 6,932)
With fecal culture in series	1,120 (747, 16,723)	908 (724, 10,000)	813 (688, 7,427)
Fecal culture			
Alone	1,623 (965, 12,292)	1,008 (810, 9,357)	839 (726, 6,640)
Medium herds (~140 cows >2 yr of age)			
Serum ELISA			
As part of the national brucellosis surveillance program			
Alone	1,678 (855, 39,378)	865 (729, 5,632)	752 (687, 4,599)
With fecal culture in series	1,258 (845, 34,483)	883 (779, 2,609)	809 (745, 1,955)
Otherwise			
Alone	1,971 (931, 47,735)	904 (746, 6,204)	767 (698, 5,210)
With fecal culture in series	1,736 (940, 66,733)	943 (808, 4,299)	832 (761, 3,033)
Milk ELISA			
As part of routine milk recording			
Alone	1,762 (867, 35,615)	869 (728, 5,672)	750 (687, 3,209)
With fecal culture in series	1,220 (825, 29,696)	880 (773, 2,645)	807 (744, 2,105)
Otherwise			
Alone	1,873 (892, 32,954)	883 (735, 6,468)	755 (690, 3,797)
With fecal culture in series	1,343 (866, 39,556)	898 (787, 3,352)	814 (749, 2,369)
Fecal culture			
Alone	2,983 (1,117, 34,123)	1,061 (826, 9,059)	837 (762, 8,176)

[1]Assuming €1 = $1.10328.

yr. In contrast, testing is conducted on all animals and the cost is roughly fixed. The testing cost per true positive detected is therefore inversely proportional to the number of positives. Early in infection, fewer animals are truly infected, and, as they are at an earlier stage of infection, fewer give positive results, giving a higher cost per positive. At all time periods except at 5 yr after MAP introduction, the total cost per true positive detected was remarkably similar, regardless of herd size, years since introduction of MAP infection, and testing strategy (Table 6). For several reasons, the results concerning total cost per true positive detected need to be interpreted with caution. Total cost per true positive detected is calculated at a single round of testing, but without considering the effect of this testing strategy on infection control over the long-term. Preferred testing strategies may not be the most cost-effective using this measure, even though they maximize the detection (and therefore culling) of infected animals. As noted previously, culling costs are also overestimated.

The work is underpinned by a range of assumptions and limitations. The Marcé model, which generated input data for the current model, was used to simulate the number of animals by age class and stage of infection in small and medium herds. It was assumed that no on-farm MAP controls were in place, with the potential for rapid within-herd transmission and high within-herd prevalence some years after initial MAP introduction (Marcé et al., 2011a; also Figure 1 and Table 3). We acknowledge that the figures are higher than often reported (Pozzato et al., 2011; Raizman et al., 2011), which may be due to ongoing interventions that are either JD-specific or that inadvertently control JD. Nonetheless, care is needed when evaluating point estimates of (apparent) prevalence from infected farms to ensure that these are compared with simulated apparent (and not true) within-herd prevalence. In the current study, we focused solely on the periods 5 to 15 yr after initial MAP introduction, as beyond this point it is implausible that no intervention would take

place. In the Marcé model, animals not infected at 1 yr of age were assumed resistant to MAP infection. This assumption is at variance with recent work (Windsor and Whittington, 2010; Espejo et al., 2013), which suggests a low but nonetheless significant number of acquired infections in animals greater than 1 yr. In the original work described by Marcé et al. (2011a), a series of sensitivity analyses were conducted, including changes to the maximum age at which animals were susceptible (varying from 4 to 18 mo). Importantly, the dynamics of infection in the herd did not change with an increase in this maximum age of susceptibility. A very high proportion of the new infections occurred in the first months of age, with new infections at a later age having no influence on the overall dynamics in the herd. In this model, a time-step of 1 wk was considered as the best compromise between precision of the transmission assumptions and simplicity of the model. Due to the nature of the output from the Marcé model, analyses at each period (5 to 15 yr after introduction of MAP infection) were restricted to those iterations where MAP prevalence was nonzero. This is consistent with the earlier finding that spontaneous fadeout occurred in 66% of runs, either in the first 2 yr (43% of runs) or subsequently (23% of runs; Marcé et al., 2011a). Spontaneous fadeout is assumed to occur as a consequence of insufficient within-herd transmission to establish infection, highlighting the importance of continual high standards of animal husbandry and management in minimizing the likelihood of infection when it is unknowingly introduced.

In the current study, we only included those assays with published Se and Sp figures at each stage of infection. For this reason, pooled culture and PCR were not included; however, the model can be easily extended as additional test data become available. As an example, it is anticipated that serum and milk ELISA will be used in series with PCR, rather than fecal culture, once robust estimates of the Se and Sp of PCR are available for each stage of infection. Although very conservative, the estimates for test Se reflect current international understanding. The mean Se estimates for the serum and milk ELISA (0.47 and 0.41, respectively; Nielsen and Toft, 2008) were consistent with earlier reports by van Weering et al. (2007), who reported a relative sensitivity of milk compared with serum ELISA with SI[infected] cattle of 0.87. In contrast, the median estimates (0.37 and 0.41, respectively; Nielsen and Toft, 2008) are not biologically plausible. For this reason, mean estimates of test Se from Nielsen and Toft (2008) were used throughout. We also used stage of infection as the basis for differences in test Se. This approach was possible, given the model output available (Marcé et al., 2011a,b). In similar work conducted previously, adjustment was also made for variation in test Se, but by age (Sergeant et al., 2008) or parity (Norton et al., 2010) rather than stage of infection. No adjustment for test Se was made with stage of lactation (and perhaps constitution of milk), although we acknowledge that this may influence milk ELISA results (Nielsen and Toft, 2012). Handling and storage of blood samples can influence serological test results (Alinovi et al., 2009), highlighting the need for standardization of sample collection and handling. In the current study, we used a Sp of 1.0 (with a range of 0.996–1.0) for fecal culture, based on the earlier work by Vidal Diez et al. (2009; the best-fitting Bayesian model for Sp of individual liquid culture, using informative priors). A most likely value of 1.0 was chosen, given our assumption (consistent with current approaches in Ireland) that positive fecal culture will be confirmed using molecular techniques. In such cases, false positives with PCR-confirmed culture are only likely because of cross-contamination, either at sampling or in the laboratory. False-positive results (and therefore a Sp estimate of less than 1.0) are likely due to passive fecal shedding of MAP in uninfected animals when using quantitative PCR in infected environments (Kralik et al., 2014). However, a similar reduction in test Sp has not been described with culture, most likely as a consequence of the significant effect of the necessary treatment of samples to reduce the level of other competing organisms in the test matrix for culture.

Several different approaches, of varying complexity, have been used to estimate the increased costs associated with premature culling. Raizman et al. (2009) calculated the total lifetime depreciation in the value of an average cow based primarily on the costs of heifer replacement and salvage value of the cow at culling, as was done here, but also took account of projected losses in lifetime milk production as a result of premature culling. Groenendaal and Wolf (2008) and Pillars et al. (2009) used an economic model to calculate the retention payoff, this being the total additional expected profit if a cow is kept until her optimal age as compared with her immediate replacement. In the current study, we focused on replacement, rather than opportunity costs, with respect to heifer replacement. Further, we assumed that replacement heifers were available and that cows were culled at the end of lactation during the parity of interest. No account was taken of the herd with respect to culling, noting that culling costs will be higher in herds where the culling rate is already high. Very limited data were available about JD and weight loss, and none that directly equates to the stages of infection. Vázquez et al. (2012) quantified weight loss by pathology, being 12.4% for seropositive animals and 26% for animals with diffuse granulomatous pathology.

In the current study, it is assumed that SI[affected] animals, being those with higher weight loss, would not go for human consumption. Given this, and in the absence of any other data, it seems reasonable to use a 0% reduction for SI[infected] animals and a 12.4% reduction for SI[infectious] animals.

Considerable uncertainty exists about the performance of MAP diagnostic tests. As outlined previously, our estimates were based on the review by Nielsen and Toft (2008), supplemented by subsequent relevant peer-reviewed publications. During the sensitivity analyses, we assumed that the Se and Sp ranges were robust, as these were the maximum and minimum values reported in the literature. For this reason, we only altered the most-likely value (to either increase or decrease the Se, to decrease the Sp). The effect of these changes on the testing and the total cost per true positive detected was relatively small (Supplementary Tables S2 and S3; http://dx.doi.org/10.3168/jds.2014-8211). As expected, cost-effectiveness decreases across all test strategies with a global decrease in Se, and vice versa. Some decrease in testing cost-effectiveness was observed with increased culling costs, which is to be expected given by the greater relative cost of culling compared with testing (Table 5).

Our study focused on testing strategies to identify infected animals at a single round of testing within dairy herds known to be infected with MAP. The effect of sequential testing is not considered here. Care is also needed if seeking to extrapolate study results to particular regions in Europe, including Ireland. First, herd demographics and management were considered typical of a western European dairy herd, including all-year calving and the sale of male calves before 4 wk of age, and may not necessarily be reflective of dairy management in specific European regions. The model was constructed, assuming introduction of infection following the introduction of a single SI[infectious] heifer. In Ireland, considerable variation exists between farms with respect to the number of animals introduced each year, and infection risk is associated with both bulls (introduced on many farms, but in small numbers) and cows (introduced on fewer farms, but in larger numbers). Further, culling rates and costs of culling will also vary between regions.

CONCLUSIONS

This study was conducted to provide robust evidence to support the use of cost-effective testing strategies in infected herds, informed by test methods, herd sizes, and costs that are relevant to the Irish industry. The key findings should be of wider relevance and interest. As expected, the detection fraction (the proportion of MAP-infected animals in the herd that are detected) was low regardless of the testing strategy used, reflecting the lower Se of current testing strategies. Culling costs were much greater than testing costs, increasing substantially with increased time since MAP introduction. Cost-effectiveness, measured according to testing cost per true positive detected, varied by testing strategy and was greater at 5 yr, compared with 10 or 15 yr, since MAP introduction. Many previous studies have highlighted the importance of early identification of infected animals and changes to herd management over the long-term to limit ongoing transmission (and further infected animals). Future work is needed to evaluate these testing strategies over time while accounting for different herd dynamics and different levels of herd biocontainment.

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