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A new compartmental model of *Mycobacterium avium* subsp. *paratuberculosis* infection dynamics in cattle

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Abstract

Models of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), a chronic infectious agent of cattle, are used to identify effective control programs. However, new biological findings show that adult infections occur and that infected animals can be separated into 2 paths: animals that will become high-shedding and, eventually, experience clinical disease (high-path); and animals that will shed only small quantities of MAP and will remain subclinical (low-path). Longitudinal data analysis found that high-path animals progress more quickly than previously believed. A standard model of MAP transmission in dairy herds was modified to include adult low-path infections and 2 infection pathways for infected calves. Analysis of this model showed that adult infection may play an important role in MAP dynamics on a dairy farm, and that the increased rate of progression for high-path animals influences both the prevalence and the persistence of MAP on a dairy farm. This new model will be able to determine the effectiveness of MAP control programs more accurately than previous models.

Keywords

paratuberculosis; disease modeling; mycobacteria; persistence; control

Introduction

Infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is responsible for Johne's disease, a chronic wasting disorder that causes a decrease in milk production (Kudahl et al., 2004; Nielsen et al., 2009; Smith et al., 2009) and pregnancy rates (Smith et al., 2010; Vanleeuwen et al., 2010) and that increases culling rates (Smith et al., 2010). The

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cost of MAP in a dairy herd is high, but the cost of control can also be high (Cho et al., 2013).

Most MAP control programs focus on hygiene to prevent exposure of calves (Benedictus and Kalis, 2003; Benedictus et al., 2000; Cho et al., 2012; Groenendaal and Galligan, 2003), as adult infection has long been thought to be rare or nonexistent (Sweeney, 1996). New analysis of longitudinal data from endemic herds, however, indicates that adult infections may occur (Espejo et al., 2013), especially when the adult is housed with a supershedder (Pradhan et al., 2011). These data also seem to indicate 2 paths for MAP infection: intermittently low-shedding or non-shedding animals that do not progress to clinical disease, and continuously shedding animals that quickly progress to high shedding and, eventually, clinical disease (Mitchell et al., 2015a, 2015b; Schukken et al., 2015). The cause of these 2 paths is unknown, but may be related to the age at infection (Mitchell et al., 2012), the dose of infection (Weber et al., 2010), the strain of infection (Mitchell et al., 2015b; Stewart et al., 2007), or genetic resistance (Gonda et al., 2006; Hinger et al., 2008). Other data sets have shown that high-shedding animals may become fecal culture (FC) positive earlier in life (Nielsen and Ersbøll, 2006). Current models of spread in herds, however, have not treated MAP as a dual-path infection (Marcé et al., 2010). Other mycobacterial diseases, though, have been successfully and usefully modeled as dual-path infections (Dye and Williams, 2008; Gomes et al., 2012; Liao et al., 2013; Meima et al., 1999). In this study, we propose a model of MAP that accounts for both calf and adult infection and that allows for dual-path infections. We examine the sensitivity of the model to assumptions, biological parameters, and potential control strategies. This model will be useful for examining the specific effects of different potential control measures.

Materials and Methods

Model structure

The proposed stochastic model for MAP transmission in cattle, adapted from Mitchell et al. (2008), is shown in Figure 1. Briefly, calves may be born susceptible (S_I) or may be vertically infected at rate γ and born transiently shedding (T_I). Susceptible calves may be infected at rate λ_I to become transiently shedding (T_I) or age out to become susceptible heifers (S_2) that age into susceptible adults (S_3). Transiently shedding calves age to transiently shedding heifers (T_2), which transition to latent heifers (E_2). Latent heifers age into latent adults on the high-shedding path (E_H) with probability η or into the low-shedding path (E_L) otherwise. Latent adults on both paths become low-shedding adults (L_H, L_L), but only animals in L_H progress to high-shedding (H), which is defined as an animal with fecal culture (FC) of ≥ 50 cfu/g. Meanwhile, susceptible adults may enter the low-shedding path in the latent category at rate λ_3 . The vertical transmission rates are determined by the number of infected adult animals, such that

$$\gamma = \mu_b (\gamma_E (E_H + E_L) + \gamma_L (L_H + L_L) + \gamma_H H) / N_3$$

where μ_b is the birth rate of the herd and N_3 is the number of adult animals in the herd. This vertical transmission rate is meant to capture true vertical transmission and post-calving horizontal transmission between calf and dam. The force of infection for calves, previously defined (Mitchell et al., 2015b), is summarized as

$$\lambda_1 = \beta (T_1 + T_2 + L_L + L_H + H)$$

where β is the rate of horizontal transmission. Adult cows contribute to the force of infection for calves as contamination of calf areas (especially calving areas) from adult cow manure is known to be a common problem (Doré et al., 2012). However, contamination of adult areas with calf feces is unlikely and, as heifers are usually managed in separate areas, contamination of heifer areas by calves or adults is also unlikely. Therefore, the force of infection for heifers and adults would rely only on direct transmission from heifers and adult animals, respectively, such that

$$\begin{aligned}\lambda_2 &= \beta_a T_2 \\ \lambda_3 &= \beta_a (L_L + L_H + H)\end{aligned}$$

where β_a is the rate of horizontal transmission to heifers and adults.

Model-specific parameters were determined by path using data from the Regional Dairy Quality Management Alliance (Pradhan et al., 2009). Briefly, 3 dairy herds in the northeast United States were followed intensively for 10 years (2004 to 2014), with all health and culling events recorded. Fecal material was collected from all adults in the herd biannually and cultured for MAP using the 4-tube fecal culture method (Pradhan et al., 2009). Additional samples (serum, feces, intestinal epithelium, and intestinal lymph nodes) were collected from the majority of cows sent to slaughter and most animals that died on-farm, and fecal and tissue samples were also cultured for MAP using the same method. For purposes of this study, animals were defined as high-path if any FC result or tissue culture result had at least one tube with >50 cfu. Animals that were not defined as high-path were defined as low-path if any FC or tissue culture result was positive. These data have previously been used to identify the two paths, labeled progressors (high-path) and non-progressors (low-path) (Schukken et al., 2015).

The length of latency in adult cattle (E_H and E_L) was defined as the period of time between first calving and first low-positive test. Animals in the high path with no low-positive results were assumed to become low shedding 90 days prior to a high-positive result, as that represented the approximate midpoint between the last negative test and the first high-positive test. The duration of low-shedding in high-path animals was defined as the period of time between the first low-positive result and the first high-positive result. Animals with positive tissue culture were assumed to be censored at the time of culling. Only animals for which all data points mentioned above (first calving date, date of positive FC or tissue culture) were included in the analysis. Rates were determined using an exponential model with interval censoring as calculated by the *survive* package in R.

We used the Gillespie algorithm to implement the stochastic model (Gillespie, 2001). Briefly, this is a continuous-time method in which both the time and identity of each event to occur are drawn from random distributions dependent on the instantaneous probability of the events. The possible events for this model and the equations used to calculate their probabilities are listed in the appendix. The model was populated by introducing 1 latent adult into an analytically-determined steady-state disease-free population (Lu et al., 2010). The model was run for 100 years over 1000 iterations and final distribution of animals from iterations in which fadeout did not occur (infection was present in at least one animal) were adopted as initial population sets. These population sets were selected with a uniform random process during model analysis.

As the Gillespie algorithm works on a continuous time scale, the interval of the approximately annual testing was allowed to vary slightly. Test-based culling was assumed to happen immediately after the first event to occur at least 1 year after the previous round of testing. Four strategies were considered: no testing, fecal culture (FC), ELISA, and fecal direct PCR. Test sensitivity for each shedding category and test specificity (Table 1) are based on a weighted average of previously published estimates. Tests were assumed to not be able to detect latent individuals, such that test-positive latent animals were a result of imperfect specificity. Only adult animals (S_3 , E_H , E_L , L_H , L_L and H) were tested. The number of test-positive animals at any given time point was calculated as

$$positive = \sum_{shedding} Pois(N_s, Se_{s,t}) + \sum_{non-shedding} Pois(N_n, Sp_t)$$

where N_s was the number of adult animals in shedding category s (L or H), $Se_{s,t}$ was the sensitivity of test t to shedding category s , N_n was the number of adult animals in non-shedding category n (S_3 or E), and Sp_t was the specificity of test t . Test-based culling was capped at 5% of the full herd size (approximately 10% of milking animals) per year, as larger test-based culling numbers would not be economically feasible; a multinomial distribution was used to select the test-positive animals to be culled, with probabilities based on the number of test-positive animals in each category. In the FC scenario, high-shedding animals were preferentially culled. Culled animals were removed from the herd after a specific delay, depending on the testing strategy's turn-around time: FC with a 3-month delay, ELISA with a 1-month delay, and fecal direct PCR with a 1-month delay.

The model was run for 25 years over 1000 iterations for each control strategy with herds of 100 total head, starting from the equilibrium distributions determined previously. The model was programmed in R 3.0.3 (The R Development Core Team, 2014), which was accessed through the Revolution R Analytics interface (© 2014 Revolution Analytics, Inc.).

Model Analysis

Outcomes considered over time were: the true prevalence of MAP infection,

$$P = (T_1 + T_2 + E_2 + E_H + L_H + H + E_L + L_L) / N$$

the probability of fadeout within 25 years ($P = 0$), and the true prevalence of shedding individuals,

$$S = (L_H + L_L + H) / N.$$

As the exact relationship between the indirect transmission coefficient for calves and adults (representing the relative susceptibility of calves and adults) is unknown, a range of scenarios were considered. In the first, $\beta_A = 0$, no adult infections were allowed. Following scenarios allowed for different ratios of β and β_A : 20:1, 10:1, 5:1, 2:1, and 1:1, as a higher susceptibility in calves than in adults has been suggested in multiple studies (Windsor and Whittington, 2010). The values of β were selected to produce approximately 10% mean shedding prevalence in endemic herds; these values are shown in Table 2.

To assess the effect of the proposed additions to MAP modeling, the results were compared to an identical analysis of 2 different models: $\eta = 1$ (only one path), and $\eta = 0.335$ (the proposed 2-path model). For the models with only one path, the transition rate from latent to low-shedding was determined as above, combining the observations for low- and high-path animals. Values of P and S and the number of low- and high-shedding animals present at 25 years were compared for all control scenarios.

Global sensitivity analysis on all parameters was performed for P and S after 5 years and for the probability of fadeout over 25 years in order to determine the most influential parameters. All parameters were sampled with a Latin Hypercube design, with all parameters sampled from uniform distributions based on the range reported (Table 2) in the literature. For test performance parameters, where multiple sources reported both results and number tested (including multiple tests reported in a single source), sensitivity and specificity values were determined using a weighted summary statistic from the *meta* package in R (Schwarzer, 2014). Partial rank correlation coefficients were calculated for all parameters for each outcome, and results were considered significant at the $\alpha = 0.05$ level with Bonferroni's correction.

Results

The average time spent in the latent category, based on the observed herds, was 1.5 years (95% CI: 1.2 to 1.8) if path was not considered. For low-path animals, average latency was 1.9 years (95% CI: 1.5 to 2.3). For high-path animals, average latency was 0.04 years (95% CI: 0 to 0.6) and average time spent low-shedding was 0.92 years (95% CI: 0.5 to 1.3). If path was not considered, the average time spent low-shedding was 0.6 years (95% CI: 0.4 to 0.8).

The true prevalence in simulated endemic herds, assuming calves had a susceptibility 20 times higher than adults, the median true prevalence was 0.21 (range: 0.08 to 0.33) and

consisted on average of 3.7 high-path animals, 2.7 of which were high-shedding at any time, and 7.2 low-path animals, with 0.8 animals infected as an adult, on average. By contrast, assuming adults and calves had equal susceptibility resulted in a slightly lower prevalence, ranging from 0.06 to 0.30 with a median of 0.19 and consisting, on average, of 1.8 high-path animals, 1.3 of which were high-shedding at any time, and 12.4 low-path animals, 8.8 of which were infected as adults. Table 2 shows the results of the models with and without adult infection or 2 infection pathways at 10 and 25 years after control implementation. Models with only 1 infection pathway required higher values of β to produce the same median shedding prevalence, and predicted higher mean numbers of high-shedding animals.

This model did not predict that these herds would be able to reliably eliminate infection using only test-and-cull control programs within 10 years (Table 2). Models assuming adults were susceptible to infection predicted a higher probability of fadeout or elimination. The probability of fadeout within 10 years was not meaningfully affected by the assumption of 2 infection pathways. However, a greater proportion of herds was able to eliminate infection using test-and-cull control programs within 25 years, especially if FC or PCR were used. The probability of fadeout using ELISA was predicted to be higher if only 1 infection pathway was assumed. The average number of high-shedding animals was predicted to be <1 after 25 years regardless of model assumptions and after 10 years in models with 2 paths or high adult susceptibility, as long as control was implemented.

Figure 2 shows the number of infected adults in each group (low path, high path, and adult infection), by testing strategy, at 3 time points (5, 10, and 25 years) after control was initiated, calves were 5 times as susceptible as adults and assuming 2 infection pathways. The total number of infected adults in the low path was predicted to be higher than the total number of infected adults in the high path without testing. In this scenario, half the low path infected animals were infected as adults. All testing strategies were able to reduce the average number of adults in each infected path, and PCR and FC were more effective than ELISA at all time points. Figure 3 shows the same values for the model assuming that calves were more susceptible than adults ($\beta_A = \beta/20$). The total number of animals in the high path were predicted to be much higher than the total number of animals in the low path, and the number of adult infections was predicted to be low. All testing strategies were able to reduce the average number of adults in each infected path, with adult infections almost eliminated after 25 years.

Table 3 shows the results of the global sensitivity analysis. In all cases high transmission rates increased prevalence, and in the absence of control a high adult culling rate resulted in decreased prevalence. The proportion of infected calves entering the high-shedding path, η , was directly and strongly correlated with shedding and true prevalence. An increase in the rate of leaving latency on the low path (σ_L) was a highly significant driver of SP and TP with no control. In cases implementing test-and-cull programs, an increase in test specificity (Sp) greatly decreased the prevalence, as it allowed the limited culling to be focused on true positives. Increases in the rate at which calves became heifers (ρ_I) and the rate at which transiently shedding heifers become latent (ϕ) resulted in significant decreases in TP, most likely due to the role of transiently shedding calves in maintaining infection, and in SP in the presence of control. The rate of vertical transmission from latent and low-shedding animals

(γ_E and γ_L) were not significantly related to total prevalence, but were related to a decrease in the probability of fadeout in the presence of control programs. Increases in the rate at which low-path animals left latency (σ_L) and the rate at which high-path animals became high-shedding (ν) significantly decreased the probability of fadeout under FC and PCR programs, as the contribution of shedding animals to transmission apparently outweighed the higher test sensitivity towards those animals. An increase in the transmission rate for adults (β_a) was associated with a significant increase in the probability of fadeout in herds using ELISA.

Discussion

This research suggests that MAP infection dynamics in a dairy herd may be influenced by the separation of infections into low and high pathways and by the possibility of adult infection. The ability of a test-and-cull program to reduce the effects of MAP depends in part on the true nature of these two factors.

Mycobacterial infections of humans (tuberculosis and leprosy) have been shown to be well represented by dual-path models, which are thought to be related to genetics and to social factors (Meima et al., 1999; Murray and Salomon, 1998). Although social factors are not an issue for cattle, environmental contamination varies between pens (Smith et al., 2011) and could correspond to such pathways, as some animals may receive a higher infectious dose than others. Genetic factors are also known to be an issue in MAP susceptibility (Koets et al., 2010, 2000), and immune competence has been suggested as a mechanism for observed differences in MAP infection dynamics (Mitchell et al., 2015a). Some of these genetic factors may be explained better using an agent-based framework, which could account for inheritance of susceptibility; however, we believe that the stochastic model framework allows us to capture the dynamics of the system.

This dual-path infection model allows for consideration of the relative importance, compared to adult infection, of the different paths in maintaining the infection. MAP has proven difficult to eliminate, despite removal of high-shedding animals and careful hygiene for calves. In our analysis, we found that adult infection may play a more important role in the dynamics of MAP prevalence in a herd than the multiple pathways, indicating that hygiene in the adult areas of the herd may also be important; this role will depend on the relative frequency of adult and calf infections, which is currently unknown. In the scenario in which susceptibility of calves was 20 times greater than adults, 13% of infected adults were high-shedding, on average, at any point in time; if calves and adults were equally susceptible, the proportion dropped to 7%, which is closer to the observation that 7% of infected animals were eventually high shedding (Mitchell et al., 2015a). This indicates that the susceptibility of adults could be closer to that of calves than previously thought, although those results depend on the full parameterization of the model. Further analysis of longitudinal data, especially using the tools of molecular epidemiology, is needed to distinguish between adult and calf-acquired infections and to increase the data available for model parameterization.

The presence or absence of the multiple pathways, on the other hand, better explained the low numbers of high-shedding animals observed in well-managed herds. In previous analysis of the longitudinal database used for calculating disease progression rates, we found averages of 0 to 0.27 high-shedding animals per pen in the 3 herds over time (Smith et al., 2011). Compared to the, on average, 0.15 to 0.8 high-shedding animals predicted per herd by the model with separate paths and the, on average, 0.4 to 1.4 high-shedding animals predicted per herd by the model without separate paths, 10 years after implementation of control, it appears that the model with separate paths better explains the observation of low numbers of high-shedding animals. Assuming that not all infections lead to high shedding resulted in lower true prevalence and higher probability of fadeout, indicating that previous models may have been overly pessimistic.

We assumed that adult infections would not lead to the high-shedding path, which would limit the importance of these infections to the overall transmission of MAP in a herd. Although studies have noted a low probability of clinical JD in animals exposed as adults (Espejo et al., 2013), few such animals will be retained in the herd long enough for high shedding and clinical disease to develop. As the probability of clinical JD in adult infections was found to be related to the amount of time exposed to MAP and the age at first exposure (Espejo et al., 2013), and faster progression to high shedding has been observed in animals that begin shedding later in life (Mitchell et al., 2015a), further studies with an agent-based modeling framework capable of capturing these dynamics are warranted. Global sensitivity analysis showed that the transmission parameters were the most important to total prevalence, regardless of control scenario, highlighting the importance of hygiene to MAP control. Vertical transmission was less important to prevalence, but was important to the probability of fadeout, and so may be able to maintain the infection under strict control programs. Test specificity was also highly important to both total and shedding prevalence. To represent the actual situation of commercial herds, which are not able to cull large proportions of the milking adults for a single cause, we limited the number of culls due to test-positive status to 10% of the milking herd each year. In such circumstances, when not all positive animals can be culled, it is more important to focus culling on the animals contributing to infections. It is possible that more frequent testing and higher culling rates would improve the probability of elimination within 10 years, but that is unlikely to be realistic in a commercial dairy herd.

The consideration of different adult susceptibilities compared to calves allowed us to examine the range of the possible situations. Although many experimental studies have shown that adults are not as susceptible to infection as calves (Windsor and Whittington, 2010), these studies have suffered from small sample sizes and limited time frames, making determination of adult susceptibility difficult. Thus, we chose to consider a range between a much lower susceptibility for adults, which we consider to be more likely, and an equal susceptibility between calves and adults, which we consider to be a worst-case scenario. This enables us to examine the importance of adult susceptibility. If adults were more susceptible, the distribution of the true prevalence in endemic herds was predicted to be lower, as was the number of high-path (and therefore high-shedding) animals. This indicates that a low adult susceptibility requires a higher true prevalence to maintain the infection in the population.

This new model will be useful for determining the effectiveness of control programs. As it explicitly includes adult infection, the effects of calf-area hygiene will not be overstated and the effects of adult-area hygiene and adult grouping can be considered. Additionally, the 2-path model allows the separation of animals that will become clinically affected, and suffer a large production impact (Smith et al., 2010, 2009), from animals that will remain subclinical and may not experience any production effects. This will be essential for economic analysis of MAP control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Adult susceptibility and dual-path infections were added to a model of MAP
- Infection in high-path animals progressed more quickly than previously believed
- Adult susceptibility can decrease the likelihood of MAP elimination on a farm
- Fast-progressing high-path animals increase MAP persistence on a farm
- Elimination of MAP from a dairy farm using a test-and-cull program is unlikely

Figure 1.
Schematic of proposed model for *Mycobacterium avium* subsp. *paratuberculosis* in cattle herds

Figure 2.

Total number of adult cows in a 100-head dairy herd endemically infected with *Mycobacterium avium* subsp. *paratuberculosis*, by test used in an annual test-and-cull strategy, at 3 time points, assuming that calves are 5 times more susceptible to infection than adults. Orange (bottom) bars are high-path animals that will eventually progress to clinical disease, yellow (middle) bars are animals infected as adults, and blue (top) bars are low-path animals that will not progress to clinical disease. The graphs represent the time points of 5, 10, and 25 years, from top to bottom.

Figure 3.

Total number of adult cows in a 100-head dairy herd endemically infected with *Mycobacterium avium* subsp. *paratuberculosis*, by test used in an annual test-and-cull strategy, at 3 time points, assuming that calves are 20 times more susceptible to infection than adults. Orange (bottom) bars are high-path animals that will eventually progress to clinical disease, yellow (middle) bars are animals infected as adults, and blue (top) bars are low-path animals that will not progress to clinical disease. The graphs represent the time points of 5, 10, and 25 years, from top to bottom.

Table 1Parameters used in a model of *Mycobacterium avium* subsp. *paratuberculosis* infection in a dairy herd.

Par.	Description	Value	Source
Se_H^E	Sensitivity of ELISA for high-shedders	0.78 (0.68–0.86)	(Clark et al., 2008; Sweeney et al., 2006)
Se_L^E	Sensitivity of ELISA for low-shedders	0.24 (0.19–0.30)	(Clark et al., 2008; Sweeney et al., 2006)
Se_H^{FC}	Sensitivity of FC for high-shedders	0.9 (0.75–1)	(Collins et al., 2006)
Se_L^{FC}	Sensitivity of FC for low-shedders	0.5 (0.25–0.75)	(Whitlock et al., 2000)
Se_H^P	Sensitivity of PCR for high-shedders	0.84 (0.77–0.90)	(Clark et al., 2008; Leite et al., 2013; Taddei et al., 2004)
Se_L^P	Sensitivity of PCR for low-shedders	0.47 (0.41–0.54)	(Clark et al., 2008; Leite et al., 2013; Taddei et al., 2004)
Sp^E	Specificity of ELISA	0.97 (0.91–0.99)	(Meens et al., 2014; Sweeney et al., 2014; Vitale et al., 2014; Whitlock et al., 2000)
Sp^F	Specificity of FC	0.98 (0.92–1)	(Sweeney et al., 2014; Vitale et al., 2014)
Sp^P	Specificity of PCR	0.94 (0.87–1)	(Taddei et al., 2004; Vitale et al., 2014)
μ_1	removal rate of calves (/year)	0.09	(Mitchell et al., 2015)
μ_2	removal rate of heifers (/year)	0.01	(Mitchell et al., 2015)
μ_3	removal rate of adults (/year)	0.35	(Mitchell et al., 2015)
μ_b	birth rate of female calves (/year)	0.45	(Mitchell et al., 2015)
γ_E	proportion of calves from latent animals infected at birth	0.01	(Mitchell et al., 2015)
γ_L	proportion of calves from low-shedding animals infected at birth	0.04	(Mitchell et al., 2015)
γ_H	proportion of calves from high-shedding animals infected at birth	0.04	(Mitchell et al., 2015)
ρ_1, ρ_2	aging rate (/year)	1	Assumed
η	proportion of animals infected as calves becoming high-shedding	0.335	(Mitchell et al., 2015)
ϕ	transition rate from transient shedding (/year)	2	(Mitchell et al., 2015; Rankin, 1961)
σ_L	transition rate from latent to low shedding, low path (/year)	0.53	Calculated
σ_H	transition rate from latent to low shedding, high path (/year)	21.5	Calculated
σ	transition rate from latent to low shedding, all animals (/year)	0.66	Calculated
ν_H	transition rate from low to high shedding, high path (/year)	1.08	Calculated
ν	transition rate from low to high shedding, all animals (/year)	1.71	Calculated
α	clinical disease-related culling rate (/year)	0.67	(Mitchell et al., 2015; Smith et al., 2010)
β	transmission coefficient	1/N, 2/N	(Mitchell et al., 2015)
β_a	transmission coefficient to adults	1/N, 0.1/N	Assumed

Table 2

Results of potential models for *Mycobacterium avium* subsp. *paratuberculosis* infection dynamics in a dairy herd. Four control programs were considered: none (N), annual fecal culture (F), annual ELISA (E), and annual direct fecal PCR (P). In test-and-cull programs, all adult animals were tested annually and test-positive animals were culled up to 10% of the herd. Transmission parameters (β and β_A) were adjusted to produce 10% median shedding prevalence in an endemically infected herd. Six models were considered: no adult infection ($\beta_A=0$), equal adult and calf susceptibility ($\beta=\beta_A$), and 4 levels of reduced adult susceptibility ($\beta_A=\beta/20$, $\beta_A=\beta/10$, $\beta_A=\beta/5$, $\beta_A=\beta/2$), each with ($\eta=0.335$) or without ($\eta=1$) separate pathways for progressing and non-progressing infections.

Years of control	Model	Parameters			Median True Prevalence			Median Shedding Prevalence			Mean Number of High Shedders			Probability of Fadeout/Elimination				
		β	β_A^2	η^3	N	F	E	P	N	F	E	P	N	F	E	P		
10	1	2.00	0	1	0.23	0.09	0.10	0.11	0.08	0.02	0.02	0.02	6.7	1.1	1.3	1.4	0.01	0.08
	2	1.80	0.090	1	0.25	0.08	0.09	0.09	0.09	0.02	0.02	0.02	6.7	1.0	1.3	1.3	0.02	0.10
	3	1.70	0.170	1	0.20	0.07	0.08	0.08	0.08	0.02	0.02	0.02	5.4	0.8	1.1	1.0	0.02	0.16
	4	1.50	0.300	1	0.18	0.05	0.07	0.06	0.07	0.01	0.02	0.02	4.3	0.6	0.8	0.7	0.05	0.25
	5	1.15	0.575	1	0.22	0.05	0.07	0.06	0.10	0.02	0.02	0.02	4.3	0.6	0.8	0.7	0.03	0.19
	6	0.80	0.800	1	0.19	0.04	0.06	0.05	0.10	0.01	0.02	0.02	3.0	0.4	0.5	0.4	0.03	0.27
	1a	1.95	0	0.335	0.25	0.10	0.14	0.11	0.10	0.03	0.04	0.03	3.4	0.6	0.8	0.7	0.01	0.07
	2a	1.80	0.090	0.335	0.21	0.08	0.11	0.09	0.08	0.02	0.03	0.03	2.6	0.4	0.6	0.5	0.02	0.12
	3a	1.75	0.175	0.335	0.23	0.08	0.11	0.09	0.09	0.02	0.04	0.03	2.7	0.4	0.6	0.5	0.02	0.12
	4a	1.52	0.304	0.335	0.21	0.07	0.09	0.08	0.09	0.02	0.03	0.02	2.2	0.3	0.5	0.4	0.04	0.15
25	5a	1.18	0.590	0.335	0.21	0.06	0.09	0.07	0.10	0.02	0.03	0.02	1.9	0.3	0.4	0.3	0.03	0.18
	6a	0.80	0.800	0.335	0.19	0.05	0.07	0.05	0.09	0.02	0.03	0.02	1.3	0.2	0.3	0.2	0.05	0.25
	1	2.00	0	1	0.24	0.06	0.06	0.06	0.08	0.01	0.01	0.01	6.7	0.3	0.4	0.4	0.04	0.61
	2	1.80	0.090	1	0.24	0.05	0.06	0.06	0.09	0.01	0.01	0.01	6.4	0.2	0.3	0.3	0.03	0.68
	3	1.70	0.170	1	0.20	0.04	0.05	0.04	0.08	0.01	0.01	0.01	5.1	0.1	0.2	0.2	0.07	0.77
																	0.69	0.75

Years of control Model	Parameters			Median True Prevalence			Median Shedding Prevalence			Mean Number of High Shedders			Probability of Fadeout/Elimination		
	β	$\beta_{A,2}$	η^3	N	F	E	P	N	F	E	P	N	F	E	P
4	1.50	0.300	1	0.18	0.03	0.04	0.04	0.07	0.01	0.01	0.01	4.1	0.1	0.2	0.1
5	1.15	0.575	1	0.22	0.03	0.04	0.03	0.10	0.01	0.01	0.01	4.2	0.1	0.1	0.1
6	0.80	0.800	1	0.19	0.02	0.03	0.03	0.09	0.01	0.01	0.01	2.8	0.0	0.1	0.0
1a	1.95	0	0.335	0.26	0.06	0.10	0.08	0.10	0.02	0.03	0.02	3.2	0.2	0.4	0.3
2a	1.80	0.090	0.335	0.22	0.05	0.07	0.06	0.09	0.01	0.02	0.02	2.7	0.1	0.3	0.1
3a	1.75	0.175	0.335	0.23	0.05	0.07	0.06	0.09	0.01	0.02	0.02	2.7	0.1	0.2	0.1
4a	1.52	0.304	0.335	0.21	0.04	0.06	0.05	0.09	0.01	0.02	0.01	2.2	0.1	0.2	0.1
5a	1.18	0.590	0.335	0.21	0.04	0.05	0.04	0.10	0.01	0.02	0.01	1.9	0.0	0.1	0.0
6a	0.80	0.800	0.335	0.19	0.03	0.04	0.03	0.09	0.01	0.02	0.01	1.3	0.0	0.0	0.0

¹Transmission rate for calves²Transmission rate for adults³Proportion of infected calves entering high (progressing) path

Table 3

Partial rank correlation coefficients from a global sensitivity analysis for mean true prevalence and mean shedding prevalence of *Mycobacterium avium* subsp. *paratuberculosis* 5 years after implementing test-and-cull control programs (None, annual fecal culture (FC), annual ELISA, and annual PCR) in an endemically infected 100-head dairy, as predicted by a stochastic model, as well as the probability of fadeout (no infected animals remaining) within 25 years. Culls were capped at 10% of the adult herd per year. Values in bold were significant at the 0.05 level with Bonferroni's correction.

Symbol	Range	Partial Rank Correlation Coefficient											
		Total Prevalence (5 years)				Shedding Prevalence (5 years)				Probability of Fadeout (25 years)			
		None	FC	ELISA	PCR	None	FC	ELISA	PCR	None	FC	ELISA	PCR
μ_1	0.08–0.1	-0.13	-0.20	-0.06	-0.02	-0.11	-0.14	-0.07	0.01	-0.12	-0.16	-0.18	-0.19
μ_2	0.008–0.015	0.00	0.11	-0.13	0.06	-0.12	0.06	-0.16	0.02	0.01	0.25	0.20	0.19
μ_3	0.3–0.4	-0.55	0.15	0.26	0.26	-0.86	0.09	0.20	0.22	0.32	0.01	-0.04	-0.04
μ_6	0.4–0.5	0.05	0.02	-0.04	0.00	-0.03	-0.08	-0.07	-0.08	-0.11	0.07	0.11	0.08
η_1	0–0.04	0.11	-0.07	-0.04	0.08	0.10	-0.01	0.00	0.13	0.07	-0.14	-0.14	-0.15
η_1	0.01–0.08	0.04	-0.01	-0.01	-0.03	0.00	-0.04	0.00	-0.04	-0.04	0.19	0.07	0.17
η_H	0.01–0.08	0.12	0.00	-0.02	-0.12	0.05	0.03	-0.09	-0.06	0.01	0.09	0.03	0.07
ρ_1	0.8–1.2	-0.78	-0.82	-0.80	-0.77	-0.19	-0.81	-0.79	-0.74	0.26	-0.13	-0.11	-0.14
ρ_2	0.8–1.2	0.28	0.35	0.31	0.26	0.42	0.38	0.34	0.31	-0.02	0.02	-0.04	-0.04
η	0.1–0.5	0.35	0.37	0.29	0.29	0.70	0.63	0.52	0.48	0.19	-0.18	-0.09	-0.12
ϕ	0.8–3	-0.64	-0.84	-0.83	-0.81	0.20	-0.82	-0.82	-0.78	0.09	0.13	0.14	0.16
σ_H	0.44–0.67	0.15	-0.02	0.05	0.03	0.19	0.04	0.15	0.05	0.03	0.00	0.02	-0.04
σ_L	1.75–40	0.40	0.00	0.22	0.05	0.66	0.21	0.31	0.11	-0.11	-0.27	-0.29	-0.23
ν	0.75–1.94	0.10	0.18	0.12	0.12	0.00	0.23	0.13	0.12	0.01	-0.14	-0.14	-0.12
α	0.5–0.8	-0.05	-0.07	-0.03	0.11	-0.12	-0.08	-0.06	0.13	-0.07	-0.01	0.02	0.01
β	0.8/N–2.1/N ¹	0.95	0.96	0.94	0.94	0.85	0.96	0.95	0.94	-0.51	-0.11	-0.07	-0.09
β_i	0–0.8/N	0.93	0.48	0.37	0.44	0.93	0.53	0.44	0.45	-0.33	0.12	0.19	0.12
Se_H	test-specific ²	NA ³	0.11	-0.16	-0.21	NA	0.06	-0.25	-0.17	NA	-0.05	-0.13	-0.06
Se_L	test-specific	NA	0.08	-0.21	0.07	NA	0.02	-0.28	0.02	NA	-0.06	0.14	0.07
Sp	test-specific	NA	-0.49	-0.70	-0.60	NA	-0.44	-0.66	-0.56	NA	0.25	0.09	-0.01

Sensitivity and specificity ranges were as appropriate to the test used (see Table 1)

N_i : the number of animals in the herd

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