Modeling the effect of test-and-slaughter strategies to control bovine tuberculosis in endemic high prevalence herds

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Abstract

Bovine tuberculosis (bTB) prevalence substantially increased over the past two decades with relatively high impact on large dairy herds, raising the concern of regulatory authorities and industry stakeholders, and threatening animal and public health. Lack of resources, together with the economic and social consequences of whole-herd stamping-out, makes depopulation an impractical disease control alternative in these herds. The increase in bTB-prevalence was associated with demographic and management changes in the dairy industry in Uruguay, reducing the efficacy of the current control program (i.e. status quo) based on intradermal serial testing with caudal fold- and comparative cervical- tuberculin test-and slaughter of reactors (CFT-CCT). Here, we aimed to assess the epidemiological effectiveness of six alternative control scenarios based on test-and-slaughter of positive animals, using mathematical modeling to infer bTB-within-herd dynamics. We simulated six alternative control strategies consisting of testing adult cattle (>1 year) in the herd every three months using one test (in-vivo or in-vitro) or a combination in parallel of two tests (CFT, interferon-gamma release assay –IGRA- or Enzyme-linked immunosorbent assay). Results showed no significant differences overall in the time needed to reach bTB-eradication (median ranging between 61 to 82 months) or official bovine tuberculosis-free status (two consecutive negative herd-tests) between any of the alternative strategies and the status quo (median ranging between 50 and 59 months). However, we demonstrate how alternative strategies can significantly reduce bTB-prevalence when applied for restricted periods (6, 12, or 24 months), and in the case of IGRAc (IGRA using peptide-cocktail antigens), without incurring on higher unnecessary slaughter of animals (false-positives) than the status quo in the first 6 months of the program (P-value <0.05). Enhanced understanding bTB-within-herd dynamics with the application of different control strategies help to identify optimal strategies to ultimately improve bTB-control and -eradication from dairies in Uruguay and similar endemic settings.

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Running title: Modeling bTB control strategies in endemic herds

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Abstract

Bovine tuberculosis (bTB) prevalence substantially increased over the past two decades with relatively high impact on large dairy herds, raising the concern of regulatory authorities and industry stakeholders, and threatening animal and public health. Lack of resources, together with the economic and social consequences of whole-herd stamping-out, makes depopulation an impractical disease control alternative in these herds. The increase in bTB-prevalence was associated with demographic and management changes in the dairy industry in Uruguay, reducing the efficacy of the current control program (i.e. *status quo*) based on intradermal serial testing with caudal fold- and comparative cervical- tuberculin test-and slaughter of reactors (CFT-CCT). Here, we aimed to assess the epidemiological effectiveness of six alternative control scenarios based on test-and-slaughter of positive animals, using mathematical modeling to infer bTB-within-herd dynamics. We simulated six alternative control strategies consisting of testing adult cattle (>1 year) in the herd every three months using one test (in-vivo or in-vitro) or a combination in parallel of two tests (CFT, interferon-gamma release assay –IGRA- or Enzyme-linked immunosorbent assay). Results showed no significant differences overall in the time needed to reach bTB-eradication (median ranging between 61 to 82 months) or official bovine tuberculosis-free status (two consecutive negative herd-tests) between any of the alternative strategies and the*status quo* (median ranging between 50 and 59 months).However, we demonstrate how alternative strategies can significantly reduce bTB-prevalence when applied for restricted periods (6, 12, or 24 months), and in the case of IGRAc (IGRA using peptide-cocktail antigens), without incurring on higher unnecessary slaughter of animals (false-positives) than the *status quo* in the first 6 months of the program (P-value <0.05). Enhanced understanding bTB-within-herd dynamics with the application of different control strategies help to identify optimal strategies to ultimately improve bTB-control and -eradication from dairies in Uruguay and similar endemic settings.

Keywords: simulation modeling, test-and-slaughter, disease control, Uruguay, *Mycobacterium bovis* , dairy cattle, disease modeling

Introduction

Mycobacterium bovis (*M. bovis*) is the primary cause of bovine tuberculosis, one of the most widespread zoonotic bacterial infections affecting cattle and other mammals (OIE 2016). Limited success in disease control has been achieved worldwide (Bezos et al., 2014; Good et al., 2018; More, Radunz, & Glanville, 2015; Morris, 2015) due to the chronic nature of the disease, the presence of wildlife reservoirs and the imperfect accuracy of currently available diagnostic strategies (Schiller et al. 2010).

The use of the tuberculin intradermal test has proven useful in detecting infected herds (Bezos et al., 2014; de la Rua-Domenech et al., 2006); however, complex host-pathogen interactions can lead to low accuracy when considering the infection status of an individual (Gormley et al., 2006; Gormley et al., 2004), challenging bTB-eradication after the establishment of infection. In herds with high prevalence (>10%), bTB-eradication with the use of the intradermal test and individual slaughter is difficult yet important, as can help to reduce pathogen circulation and consequently decrease potential zoonotic risk, and negatively impact animal health and welfare.

In bTB-high prevalence settings, two approaches to overcome the limited sensitivity of the skin tests have been used for eradication of the disease at the herd level globally. One is the use of ancillary in-vitro tests to maximize the ability to detect infected cattle in the herd (Council Directive 64/432/EEC, Casal et al. 2014), and the other is the depopulation of the herd from which individual reactors were detected (More, Radunz, & Glanville, 2015; Verteramo Chiu et al., 2019). Advantages and limitations of the use of in-vitro ancillary diagnostic tests have been reviewed elsewhere (Bezos, Casal, et al. 2014; de la Rua-Domenech et al. 2006), but essentially these tests target infected animals that may be missed by the intradermal test. Whole herd depopulation, though costly, is an effective strategy to control and eradicate bTB since it ensures disease elimination (More et al. 2015). However, the complexity of its implementation increases with the size of the bTB-infected herd, making it implausible at times, given the limited resources for the compensation to farmers for culled animals in many countries. The lack of resources, together with economic and social implications makes depopulation difficult to justify to stakeholders, especially for herds in which a low proportion of the animals test positive, as often seen in endemic settings (Ciaravino et al., 2017).

In Uruguay, bTB-control programs rely on the serial application of the caudal fold tuberculin test –CFT- followed by the comparative cervical tuberculin test –CCT- in the reactors and subsequent slaughter of CCT positive animals for confirmation of *M. bovis* infection by a bacteriological culture of selected tissues sampled at the slaughterhouse. Herds in which bTB-infection is confirmed are subjected to retesting using intradermal tests (CFT+CCT) every 60 to 180 days, until two consecutive negative results are achieved, leading to regaining the officially tuberculosis-free status (OTF) (MGAP 1989). Government indemnity is provided to farmers for those animals slaughtered under the regulations of the bTB-control program (Law 19300, 26/12/2014.DGSG/MGAP).

In the Uruguay cattle population, with the above described bTB-control program in place, a low bTB-prevalence was traditionally reported (<0.001 at the herd level) (WAHIS_OIE 2014). Within the past two decades, however, the number of bTB-infected dairy herds, the within-herd bTB-prevalence, and the time required from detection to regaining the officially tuberculosis-free status (OTF) increased (Picasso-Risso et al. 2019), leading to unprecedented challenges in the control of bTB in Uruguay. The difficulty in controlling bTB has been associated with changes in dairy demographic structure and management (Picasso et al. 2017; Picasso-Risso et al. 2019), including larger herds (>360 animals), higher animal density (DIEA 2018), increased animal movements, and more intensive animal rearing than traditional dairy farming prior to the 1990s (Picasso et al. 2017). In this context, the question is whether the current Uruguayan bTB-program is sufficient to control bTB in herds once the infection is confirmed and some level of within-herd transmission is suspected.

Mathematical models have been broadly used to understand within-herd bTB-transmission patterns and to evaluate control and surveillance strategies (Alvarez et al., 2014; Brooks-Pollock, Roberts, & Keeling, 2014; Ciaravino et al., 2018; Perez, Ward, & Ritacco, 2002; Rossi, et al., 2019). These models account for the chronic nature of bTB, considering the long and variable incubation periods, biological variabilities, and the influence of different production systems (Alvarez et al. 2014) while avoiding the risks and the costs of in-vivo implementation (Halasa and Dürr 2017). An integrated within-and between-herd model has been parameterized and validated to evaluate the performance of risk-targeted bTB-surveillance using the current test-and-slaughter bTB- strategies in Uruguay (VanderWaal et al. 2017). However, previous studies have suggested that the sensitivity of the test-and-slaughter program is impaired in high prevalence dairy herds in Uruguay (Picasso-Risso et al. 2019), and given that depopulation of these large herds is not economically, logistically, or socially feasible, the use of alternative diagnostic in-vitro assays is a reasonable alternative strategy to evaluate for control in these herds. In this study, we aimed to assess the effectiveness of different alternative bTB-control strategies, to ultimately elucidate the optimal option for control in bTB-high prevalence dairy herds in Uruguay when depopulation is not an alternative.

Methods:

Model description

Our objective was to model disease dynamics at the herd level for in dairies in Uruguay, considering herd demographics, bTB transmission, and bTB-control measures. We evaluated the relative effectiveness of the*status quo* and six alternative control scenarios in dairy herds with bTB-high prevalence (>10%) (Table 1) using a modified within-herd bTB transmission model developed and parameterized for Uruguayan cattle herds (VanderWaal et al. 2017). The outputs of the model were a) time-to-bTB-eradication, b) bTB-prevalence at six months and ten years post-infection, c) time-to-regain the OTF status, and d) proportion of animals slaughtered in the herd.

Herd demographics

We used two animal categories, adults ([?]12 months), and calves (<12 months), with calves becoming adults after reaching one year of age (rate 1/12 months) (VanderWaal et al. 2017). Slaughter, birth, and replacement of death/culled rates were assumed to follow a Poisson distribution based on demographic information available. The average proportion of routine slaughter for adults ($\lambda_{\text{sl.a}}$) and calves ($\lambda_{\text{sl.c}}$) was 0.268 and 0.007 respectively (VanderWaal et al. 2017). Then, in order to maintain a stable herd size, births and adult replacements were happening every four months ($s = 4$), with births at the same rate of calves slaughters ($\lambda_{\text{sl.c}}$), and adult replacements at similar rates as adult slaughter ($\lambda_{\text{sl.a}}$). The model was initialized with a population of 500 animals and an adult to calf ratio of 75/25 following the typical demographic characteristics of large dairy herds in the past decades in Uruguay (DIEA 2018).

Individual-based bTB-transmission dynamics

bTB-transmission was simulated using a stochastic, discrete, compartment model, in which animals transitioned through four mutually exclusive stages; susceptible (S), Occult (O), Reactive (to diagnostic tests) (R), and Infectious (I; SORI model) (Alvarez et al. 2014; Ciaravino et al. 2018; Conlan et al. 2012; Perez et al. 2002). When healthy animals from the susceptible compartment (S) are infected with *M. bovis* , they transition to the Occult (O) state for a latent period (λ_1) in which even though infected, they are not detectable by antemortem bTB tests or infectious.

As the disease progresses, occult animals become detectable by diagnostic tests and move to the sub-compartments R_a and R_b based on two different times for detection (λ_{2a} and λ_{2b}) respectively: λ_{2a} represents the period until IGRA is able to identify the infection, and λ_{2b} is the time until all the other diagnostic tests implemented can identify bTB-infected animals.

The final compartment represents animals that become infectious (I) while remain also detectable by the antemortem diagnostic tests (Figure 1a). A similar SORI-model was applied for the two age categories (adults/calves). At each time step, the number of animals that transitioned between compartments was selected from a Poisson distribution with the purpose of incorporating stochasticity to the model as previously described (Gillespie 2001; Keeling and Rohani 2008). Transition rates were based on the following deterministic backbone differential equations:

$$\frac{\mathrm{dS_{cal}}}{\mathrm{dt}} = -\left(\beta\ \frac{S_{\mathrm{calv}}(I_{\mathrm{calv}}+I_{\mathrm{ad}})}{N}\right); \frac{\mathrm{dS_{ad}}}{\mathrm{dt}} = -\left(\beta\ \frac{S_{\mathrm{ad}}(I_{\mathrm{calv}}+I_{\mathrm{ad}})}{N}\right)$$

$$\frac{\mathrm{dO_{calv}}}{\mathrm{dt}} = \left(\beta\ \frac{S_{\mathrm{calv}}(I_{\mathrm{calv}}+I_{\mathrm{ad}})}{N}\right) - \left(O_{\mathrm{calv}}\frac{1}{\lambda_1}\right); \frac{\mathrm{dO_{ad}}}{\mathrm{dt}} = \left(\beta\ \frac{S_{\mathrm{ad}}(I_{\mathrm{calv}}+I_{\mathrm{ad}})}{N}\right) - \left(O_{\mathrm{ad}}\frac{1}{\lambda_1}\right)$$

$$\frac{\mathrm{dRa_{calv}}}{\mathrm{dt}} = \left(O_{\mathrm{calv}}\frac{1}{\lambda_1}\right) - \left(\mathrm{Ra_{calv}}\frac{1}{\lambda_{2a}}\right); \frac{\mathrm{dRa_{ad}}}{\mathrm{dt}} = \left(O_{\mathrm{ad}}\frac{1}{\lambda_1}\right) - \left(\mathrm{Ra_{ad}}\frac{1}{\lambda_{2a}}\right)$$

$$\frac{\mathrm{dRb_{calv}}}{\mathrm{dt}} = \left(\mathrm{Ra_{calv}}\frac{1}{\lambda_{2a}}\right) - \left(\mathrm{Rb_{calv}}\frac{1}{\lambda_{2b}}\right); \frac{\mathrm{dRb_{ad}}}{\mathrm{dt}} = \left(\mathrm{Ra_{ad}}\frac{1}{\lambda_{2a}}\right) - \left(\mathrm{Rb_{ad}}\frac{1}{\lambda_{2b}}\right)$$

$$\frac{\mathrm{dI_{calv}}}{\mathrm{dt}} = \left(\mathrm{Rb_{calv}}\frac{1}{\lambda_{2b}}\right); \frac{\mathrm{dI_{ad}}}{\mathrm{dt}} = \left(\mathrm{Rb_{ad}}\frac{1}{\lambda_{2b}}\right)$$

We assumed that animals within the adult and calf compartments interact with equal probability (homogeneous mixing) and that transmission is frequency-dependent (Smith et al., 2013; VanderWaal et al., 2017).

Individual-based bTB-control dynamics

We compared the current control strategy (i.e. *status quo*) to six alternative scenarios applied to adult animals (>12 months) in the herd. Testing was performed every three months for all adult animals, consistent with the *status quo* control strategy in Uruguay (MGAP 1989). The alternative strategies aimed to improve the sensitivity of the control program (*status quo* testing) by using a maximum of two diagnostic tests every testing period. The six alternatives were the use of CFT only, the use of IGRA (assessing two different antigens commercially available for the region), and the parallel combination of CFT+IGRA, CFT+ELISA, or IGRA+ELISA (Table 1). For the parallel combination, the IGRA selected used peptide cocktail antigens given it demonstrated to have an improved specificity with similar sensitivity under Uruguayan field conditions (Picasso-Risso et al. 2019).

The sensitivity (Se) and specificity (Sp) of each testing strategy were modeled using different beta distributions for the different stages (Table 2) based on previous estimates (Picasso-Risso et al. 2019). Test-positive animals were immediately removed from the herd before the following testing period. To track the number of slaughtered animals we created four mutually exclusive compartments, one for the false-positives (susceptible false positives -$S_{S\text{-}}$) and three for the true-positives (reactors or infectious positives -Ra_S, Rb_S, $I_{S\text{-}}$) to each control strategy (Figure 1b). The number of reactor animals in each of these four compartments were assumed to be drawn from a Poisson distribution centered on the expected number of false-positive animals given the susceptible population (S_s), and on the expected number of true-positives in the infected compartments (Ra_s, Rb_s, I_s) using equations 1 and 2 for strategies involving one-test or parallel testing respectively. Dependency between the test results when applying two tests was introduced through positive and negative correlation coefficients ($\rho\Delta\varsigma$ and $\rho\Delta$) following the distributions described previously for Uruguay (Picasso-Risso et al. 2019).

Eq 1. False-Positives: $S_s = [1 - Sp)] * S_{\mathbf{ad}}$ **(single testing)**

$S_s = [1 - (Sp_1 * \ \mathrm{Sp}_2 \ + \ rhoD)] * S_{\mathrm{ad}}$(parallel testing)

Eq 2. True-Positives: $\mathbf{Ra}_s = Se * \mathbf{Ra_{ad}}$ **;** $\mathbf{Rb}_c = Se * \mathbf{Rb_{ad}}$ **;** $I_s = Se * I_{\mathbf{ad}}$ **(single testing)**

$\mathrm{Ra}_s = (Se_1 * \ \mathrm{Se}_2 \ + \ rhoDc) * \mathrm{Ra}_{\mathrm{ad}}$(parallel testing)

$\mathrm{Rb}_s = (Se_1 * \ \mathrm{Se}_2 \ + \ rhoDc) * \mathrm{Rb}_{\mathrm{ad}}$

$I_s = (Se_1 * \ \mathrm{Se}_2 \ + \ rhoDc) * I_{\mathrm{ad}}$

Assessment of alternative strategies

The model was run without application of any control strategy until the median apparent prevalence (the sum of the animals in compartments R and I) of 500 iterations reached 10% (high prevalence herd). Then, the median number of animals in each of the infected compartments (O-R-I) was used to seed each of the six models evaluating bTB-control strategies (Supplementary Figure S1).

Models with each control strategy were run for 500 simulations for 20 years, and results were summarized as median, and 2.5, 25, 75, and 97.5% intervals, meaning the interval containing 2.5, 25, 50, 75, and 97.5% of the outcomes. Differences between the outcomes were compared using the Kruskal-Wallis test (Kruskal and Wallis 1952), Dunn's test for pairwise comparison, and log-rank test to compare time to eradication and OTF.

Results:

Epidemiological indicators:

Estimates of the time to achieve bTB-eradication, both in the whole herd and when considering each of the age categories separately, showed slightly different medians depending on the scenario considered (Figures 2-3). The median time to eradication ranged from 61 to 82 months when considering the whole herd, and if only adults were considered, from 41 to 52 months (Table 3). Towards the end of the outbreak (when bTB-prevalence in adults reached zero), calves (which were not tested) carried most of the residual infections, maintaining the circulation of the disease for significant (Kruskal-Wallis P-value <0.05) longer periods (Figure 3).

There was a significant difference (P-value<0.05) in bTB-prevalence at the early stages of the outbreak (6, 12, and 24 months) when alternative strategies were applied compared to the*status quo* (Table 4). At the end of the second year of simulations, bTB-prevalence estimates under the *status quo* were only significantly (P-value =0.01) different from those generated when considering the CFT+IGRA strategy. Differences with estimates from the IGRAb and IGRAc scenarios were not considered significant though were close to the p-value threshold (P-value=0.22) (Table 4).

Time to regain OTF status did not vary between the strategies simulated (P-value >0.05) (Table 3), with median estimates ranging between 50 and 59 months (4.1 to 4.9 years) under all scenarios.

Performance effectiveness:

The simulated scenarios using ELISA as an ancillary test (IGRA+ELISA and CFT+ELISA) led to a higher proportion of animals testing positive, with higher rates of false-positives (0.155, 95^{th}percentile:0.120-0.191 and 0.177, 95^{th} percentile: 0.141-0.212 (Figure 4) than the *status quo* or any of the other alternative scenarios. The *status quo* scenario yielded the lowest proportion of positive diagnostic results, and the lowest estimates for the proportion of false-positive results (median: 0.08, 95_{th} percentile: 0.05-0.13), which were significantly different from all the other simulated strategies according to the Kruskal-Wallis test (P-value <0.05).

Discussion

In this study, we simulated bTB-transmission under very specific conditions (large herds and a high within-herd apparent prevalence) in a dairy cattle herd in Uruguay to try to assess the performance of current and potentially available control strategies, given that this is an emerging problem faced by animal health authorities in the country for which the effectiveness of the tools at hand has not been evaluated extensively. The fixed size of the herd (500-cattle) was considered large for the country given that it is $>75^{\text{th}}$ percentile for dairies in Uruguay (DIEA 2018; Picasso et al. 2017). Furthermore, the starting apparent prevalence of 10% was high most challenging bTB-infected herds in Uruguay (Picasso Risso 2016).

In this context, we assumed the presence of two independent populations in the herd (adults and calves), of which only the former would be subjected to every three months to seven control strategies (*status quo* and six alternatives) considered alternatively.

Alternative scenarios considered tools currently available (and applied) for bTB control elsewhere. Among these, the application of IGRAs can help in earlier detection (˜2 weeks) of the bTB- cell-mediated immune response in comparison to the intradermal test (or the ELISA) (Bezos, Casal, et al. 2014; de la Rua-Domenech et al. 2006). By including two subcompartments (Ra and Rb) in the R compartment, our analysis accounted for variations in the duration of the detection period described for the IGRAs (de la Rua-Domenech et al. 2006a). Two different protocols for the application of the IGRA where considered, based on the use of PPD or a more specific set of antigens that under the Uruguayan conditions has been proved to provide a similar Se with no loss in Sp (Picasso-Risso et al. 2019). Furthermore, high bTB-prevalence herds tend to have animals in various stages of the disease, including advanced stages, which can result in an improved Se for

antibody-based diagnostics such as ELISA (de la Rua-Domenech et al. 2006; Waters et al. 2011). Hence, ELISA was assessed as an alternative strategy to the cell-mediated diagnostics for its potential in these endemic herds.

We found there was no difference in the time to reach bTB-eradication or OTF-status between the six alternatives and the *status quo*(median 73, 36-133 months, and 59, 26-122 months respectively), although the large variability in the estimates obtained could have limited our ability to detect such differences. The relative costs associated with the slaughter of uninfected (but test-positive) animals was significantly increased with the use of most of the alternative strategies (except for IGRAc), with the highest cost performance observed with strategies including the ELISA (Supplementary Figure S2). The lack of significant improvement was associated with the maintenance of the disease in the calf category (Figure 3). In most of the simulated scenarios, eradication was reached earlier in the adult category than the calf category (Table 3), since calves remain undetected until reaching the age to be tested. Undetected calves were responsible for sustaining bTB in the herd for longer periods in most simulations (Table 3, Figure 3). We, therefore, need to consider this conclusion might not hold when simulating control strategies that include calfhood testing.

When exploring the effect of the control strategies in shorter periods (after 6, 12 and 24 months), we observed a significant reduction in bTB-prevalence after the first 6 and 12 months with the use of any of the six alternative strategies of control, and after 24 months with the use of CFT+IGRA parallel testing. The model outputs suggest that alternative strategies can be selected as an initial strategy, and then could be followed by the use of the current *status quo* strategy for eradication. In addition, when the cost of implementation of alternative strategies was assessed (including for unnecessary slaughter), the unique strategy that matched the performance of the*status quo* in the first 6 months of testing (P-value >0.05) was the use of IGRAc (Supplementary Figure S2). This finding suggests that IGRAc might be an effective tool to quickly reduce bTB-prevalence at the initial stages of the control program (six months or two consecutive tests).

The Se and Sp of the diagnostic tests considered in the different control scenarios were based on estimates of accuracy based on information from high bTB-prevalence dairy herds in Uruguay (Picasso-Risso et al. 2019), which helped reduce the uncertainty on test performance derived from the sometimes contradictory estimates described in previous studies (Alvarez et al. 2012; Bezos, Casal, et al. 2014)). Testing intervals (3 months) represent a high pressure for detection of*M. bovis* using intradermal testing to elude the anergy period described as a result of multiple intradermal inoculations (Radunz and Lepper 1985; de la Rua-Domenech et al. 2006b; Vordemeier et al. 2006), and logistically allow slaughter before the next testing period. Although in-vitro testing allows for more frequent testing and can benefit from the booster effect after tuberculin inoculation when applied in parallel (CFT+IGRA) (Casal et al. 2014; Palmer et al. 2006; Schiller et al. 2010), we chose to assess the strategies in reference to the *status quo* , and we avoided inclusion of shorter testing-intervals. We recognize that a deeper understanding of the effect of different testing periods is needed.

In order to identify optimal testing strategies, we balanced the epidemiologic effectiveness of disease control while minimizing the unnecessary culling of false reactors relative to the *status quo* . While an initial useful approximation of the additional efforts imposed by each strategy, a next step is to provide an estimation of the economic cost, including costs of testing as well as unnecessary culling of false-positive cattle (Kao, Roberts, & Ryan, 1997; Kao et al., 2018; Smith et al., 2013) and social acceptance (Ciaravino et al., 2017); both are essential before implementation.

Conclusions

In this report, we conclude that the assessed alternative strategies were not able to improve the time to bTB-eradication, time to regain the OTF-status, or were able to reduce the number of false-positive cattle. Results from this study contribute to the understanding of the implications of applying different testing pressures in highly infected dairy herds in Uruguay. Additionally, we showed the importance of targeting surveillance and control strategies to infected calves, the benefit of using the IGRAc as an ancillary test in initial stages

of control. Determination of the best testing strategy will be a result of epidemiologic, performance, and economic balance while acknowledging logistics and socio-cultural perceptions of individual countries and regions. Our results enhance understanding of bTB-within-herd dynamics and identify optimal bTB-control strategies for dairies in Uruguay and similar endemic settings.

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Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a research article with no original research data

Conflicts of interest statement

Authors declare that this research was conducted in absence of any personal, financial or commercial relationship that could result in a conflict of interest.

Data availability statement

Data sharing is not applicable to this article as no new data were analyzed in this study.

References

Alvarez, Julio, Javier Bezos, Maria Luisa de la Cruz, Carmen Casal, Beatriz Romero, Lucas Domínguez, Lucía de Juan, and Andrés Pérez. 2014. "Bovine Tuberculosis: Within-Herd Transmission Models to Support and Direct the Decision-Making Process." *Bovine Tuberculosis*97:S61–68.

Alvarez, Julio, Andrés Perez, Javier Bezos, Sergio Marqués, Anna Grau, Jose Luis Saez, Olga Mínguez, Lucía de Juan, and Lucas Domínguez. 2012. "Evaluation of the Sensitivity and Specificity of Bovine Tuberculosis Diagnostic Tests in Naturally Infected Cattle Herds Using a Bayesian Approach." *Veterinary Microbiology* 155(1):38–43.

Bezos, Javier, Julio Álvarez, Beatriz Romero, Lucía de Juan, and Lucas Domínguez. 2014. "Bovine Tuberculosis: Historical Perspective." *Bovine Tuberculosis* 97, Supplement:S3–4.

Bezos, Javier, Carmen Casal, Beatriz Romero, Bjoern Schroeder, Roland Hardegger, Alex J. Raeber, Lissette López, Paloma Rueda, and Lucas Domínguez. 2014. "Current Ante-Mortem Techniques for Diagnosis of Bovine Tuberculosis." *Bovine Tuberculosis* 97, Supplement:S44–52.

Brooks-Pollock, Ellen, Gareth O. Roberts, and Matt J. Keeling. 2014. "A Dynamic Model of Bovine Tuberculosis Spread and Control in Great Britain." *Nature* 511(7508):228–31.

Casal, Carmen, Alberto Díez-Guerrier, Julio Álvarez, Sabrina Rodriguez-Campos, Ana Mateos, Richard Linscott, Edmond Martel, John C. Lawrence, Clare Whelan, John Clarke, Amanda O'Brien, Lucas Domínguez, and Alicia Aranaz. 2014. "Strategic Use of Serology for the Diagnosis of Bovine Tuberculosis after Intradermal Skin Testing." *Veterinary Microbiology* 170(3–4):342–51.

Ciaravino, G., A. García-Saenz, S. Cabras, A. Allepuz, J. Casal, I. García-Bocanegra, A. De Koeijer, S. Gubbins, J. L. Sáez, D. Cano-Terriza, and S. Napp. 2018. "Assessing the Variability in Transmission of Bovine Tuberculosis within Spanish Cattle Herds." *Epidemics* 23:110–20.

Ciaravino, Giovanna, Patricia Ibarra, Ester Casal, Sergi Lopez, Josep Espluga, Jordi Casal, Sebastian Napp, and Alberto Allepuz. 2017. "Farmer and Veterinarian Attitudes towards the Bovine Tuberculosis Eradication Programme in Spain: What Is Going on in the Field?" *Frontiers in Veterinary Science* 4:202–202.

Conlan, Andrew J. K., Trevelyan J. McKinley, Katerina Karolemeas, Ellen Brooks Pollock, Anthony V. Goodchild, Andrew P. Mitchell, Colin P. D. Birch, Richard S. Clifton-Hadley, and James L. N. Wood. 2012. "Estimating the Hidden Burden of Bovine Tuberculosis in Great Britain." *PLOS Computational Biology* 8(10):e1002730.

DIEA. 2018. "Anuario Estadístico."

Gillespie, Daniel T. 2001. "Approximate Accelerated Stochastic Simulation of Chemically Reacting Systems." *The Journal of Chemical Physics* 115(4):1716–33.

Good, Margaret, Douwe Bakker, Anthony Duignan, and Daniel M. Collins. 2018. "The History of In Vivo Tuberculin Testing in Bovines: Tuberculosis, a 'One Health' Issue." *Frontiers in Veterinary Science* 5:59–59.

Gormley, E., M. B. Doyle, T. Fitzsimons, K. McGill, and J. D. Collins. 2006. "Diagnosis of Mycobacterium Bovis Infection in Cattle by Use of the Gamma-Interferon (Bovigam®) Assay." *4th International Conference on Mycobacterium Bovis* 112(2):171–79.

Gormley, E., M. B. Doyle, K. McGill, E. Costello, M. Good, and J. D. Collins. 2004. "The Effect of the Tuberculin Test and the Consequences of a Delay in Blood Culture on the Sensitivity of a Gamma-Interferon Assay for the Detection of Mycobacterium Bovis Infection in Cattle." *Veterinary Immunology and Immunopathology* 102(4):413–20.

Halasa, Tariq, and Salome Dürr. 2017. "Editorial: Modeling Disease Spread and Control." *Frontiers in Veterinary Science* 4:199.

Kao R. R., Roberts M. G., and Ryan T. J. 1997. "A Model of Bovine Tuberculosis Control in Domesticated Cattle Herds." *Proceedings of the Royal Society of London. Series B: Biological Sciences*264(1384):1069–76.

Kao, Szu-Yu Zoe, Kimberly VanderWaal, Eva A. Enns, Meggan E. Craft, Julio Alvarez, Catalina Picasso, and Scott J. Wells. 2018. "Modeling Cost-Effectiveness of Risk-Based Bovine Tuberculosis Surveillance in Minnesota." *Preventive Veterinary Medicine* 159:1–11.

Keeling, Matt J., and P. Rohani. 2008. *Modelling Infectious Diseases in Human and Animals.* Princeton University Press.

Kruskal, William H., and W. Allen Wallis. 1952. "Use of Ranks in One-Criterion Variance Analysis." *Journal of the American Statistical Association* 47(260):583–621.

MGAP, Ministerio de Ganaderia Agricultura y Pesca. 1989. "Legislacion Sanitaria Animal."

More, S. J., B. Radunz, and R. J. Glanville. 2015. "Lessons Learned during the Successful Eradication of Bovine Tuberculosis from Australia." *Veterinary Record* 177(9):224–32.

Morris, Roger S. 2015. "Diseases, Dilemmas, Decisions—Converting Epidemiological Dilemmas into Successful Disease Control Decisions." *Preventive Veterinary Medicine* 122(1):242–52.

OIE. 2016. "World Animal Health_OIE." http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home.

Palmer, M. V., W. R. Waters, T. C. Thacker, R. Greenwald, J. Esfandiari, and K. P. Lyashchenko. 2006. "Effects of Different Tuberculin Skin-Testing Regimens on Gamma Interferon and Antibody Responses in Cattle Experimentally Infected with Mycobacterium Bovis." *Clinical and Vaccine Immunology* 13(3):387–94.

Perez, Andres M., Michael P. Ward, and Viviana Ritacco. 2002. "Simulation-Model Evaluation of Bovine Tuberculosis-Eradication Strategies in Argentine Dairy Herds." *Preventive Veterinary Medicine* 54(4):351–60.

Picasso, C., Julio Alvarez, Kimberly L. VanderWaal, Federico Fernandez, Andres Gil, Scott J. Wells, and Andres Perez. 2017. "Epidemiological Investigation of Bovine Tuberculosis Outbreaks in Uruguay (2011–2013)." *Preventive Veterinary Medicine* 138:156–61.

Picasso Risso, Catalina. 2016. "Epidemiological Investigation of Bovine Tuberculosis Outbreaks in Uruguay (2011–2013)." University of Minnesota, Twin Cities.

Picasso-Risso, C., A. Gil, A. Nunez, A. Suanes, V. Macchi, X. Salaberry, J. Alvarez, and A. Perez. 2019. "Diagnostic Interaction between Bovine Tuberculosis (BTB) and Johne's Disease in BTB Highly Prevalent Dairy Farms of Uruguay." *Veterinary and Animal Science* 100052.

Picasso-Risso, Catalina, Andres Perez, Andres Gil, Alvaro Nunez, Ximena Salaberry, Alejandra Suanes, and Julio Alvarez. 2019. "Modeling the Accuracy of Two In-Vitro Bovine Tuberculosis Tests Using a Bayesian Approach." *Frontiers in Veterinary Science* 6:261.

Radunz, B., and Lepper A. W. D. 1985. "Suppression of Skin Reactivity to Bovine Tuberculin in Repeat Tests." *Australian Veterinary Journal* 62(6):191–94.

Rossi, Gianluigi, Pascale Aubry, Caroline Dube, and Rebecca L. Smith. 2019. "The Spread of Bovine Tuberculosis in Canadian Shared Pastures: Data, Model, and Simulations." *Transboundary and Emerging Diseases* 66(1):562.

de la Rua-Domenech, R., A. T. Goodchild, H. M. Vordermeier, R. G. Hewinson, K. H. Christiansen, and R. S. Clifton-Hadley. 2006a. "Ante Mortem Diagnosis of Tuberculosis in Cattle: A Review of the Tuberculin Tests, γ-Interferon Assay and Other Ancillary Diagnostic Techniques." *Research in Veterinary Science* 81(2):190–210.

de la Rua-Domenech, R., A. T. Goodchild, H. M. Vordermeier, R. G. Hewinson, K. H. Christiansen, and R. S. Clifton-Hadley. 2006b. "Ante Mortem Diagnosis of Tuberculosis in Cattle: A Review of the Tuberculin Tests, γ-Interferon Assay and Other Ancillary Diagnostic Techniques." *Research in Veterinary Science* 81(2):190–210.

Schiller, Irene, H. Martin Vordermeier, W. Ray Waters, Adam O. Whelan, Michael Coad, Eamonn Gormley, Bryce M. Buddle, Mitchell Palmer, Tyler Thacker, Jim McNair, Michael Welsh, R. Glyn Hewinson, and Bruno Oesch. 2010. "Bovine Tuberculosis: Effect of the Tuberculin Skin Test on in Vitro Interferon Gamma Responses." *Veterinary Immunology and Immunopathology* 136(1–2):1–11.

Smith, Rebecca L, Ynte H. Schukken, Zhao Lu, Rebecca M. Mitchell, and Yrjo T. Grohn. 2013. "Development of a Model to Simulate Infection Dynamics of Mycobacterium Bovis in Cattle Herds in the United States." *Journal of the American Veterinary Medical Association*243(3):411–23.

Smith, Rebecca L., Loren W. Tauer, Ynte H. Schukken, Zhao Lu, and Yrjo T. Grohn. 2013. "Minimization of Bovine Tuberculosis Control Costs in US Dairy Herds." *Preventive Veterinary Medicine*112(3–4):266–75.

VanderWaal, KL, Eva A. Enns, Catalina Picasso, Julio Alvarez, Andres Perez, Federico Fernandez, Andres Gil, Meggan Craft, and Scott Wells. 2017. "Optimal Surveillance Strategies for Bovine Tuberculosis in a Low-Prevalence Country." *Scientific Reports* 7(1):4140.

Verteramo Chiu, Leslie J., Loren W. Tauer, Rebecca L. Smith, and Yrjo T. Grohn. 2019. "Assessment of the Bovine Tuberculosis Elimination Protocol in the United States." *Journal of Dairy Science*102(3):2384–2400.

Vordemeier, Martin, Anthony V. Goodchild, Adam Whelan, Katie Ewer, Richard S. Clifton-Hadley, John Williams, and R. Glyn Hewinson. 2006. "The Bovigam Assay as Ancillary Test to the Tuberculin Skin Test." *Government Veterinary Journal* 16:72–80.

WAHIS_OIE. 2014. "Animal Health Situation." http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation.

Waters, W. R., B. M. Buddle, H. M. Vordermeier, E. Gormley, M. V. Palmer, T. C. Thacker, J. P. Bannantine, J. R. Stabel, R. Linscott, E. Martel, F. Milian, W. Foshaug, and J. C. Lawrence. 2011. "Development and Evaluation of an Enzyme-Linked Immunosorbent Assay for Use in the Detection of Bovine Tuberculosis in Cattle." *Clinical and Vaccine Immunology : CVI* 18(11):1882–88.

Tables and Figures legends

Table 1.

Control strategies evaluated by the model.

Table 2.

Sensitivity and specificity estimates used for modeling each bTB-testing strategy

Table 3.

Median (med), 2.5(q2.5), 25(q25), 75(q75), and 97.5 (q97.5) percentiles for the 500 simulations of the time (years and months) and the number of tests necessary to reach bTB-eradication, and to reach the officially tuberculosis-free (OTF) status for the *status quo* and the six alternative control strategies. The columns 3 to 12 show the eradication estimates for the complete herd (adults and calves), and the adult animals solely. The last five columns indicate the estimates for OTF. Colors represent four different time categories: <3 years (green), 3 to 6 years (grey), >6 to 9 years (coral), and >9 years (red) or its respective months and number of tests performed in that period.

Table 4.

bTB-prevalence estimates at the end of the 6, 12, 24 months of simulating control strategies.

Figure 1.

Diagram representing the bTB-transmission compartments (Figure 1a) including calves (top row) and adults (second row), and equations driving the control strategies dynamics (Figure 1b). The number of animals in each bTB-compartment is indicated as susceptible (S), occult (O), reactors in subgroup Ra and Rb, and infectious (I). Transmission rates between infectious and susceptible stages are represented by β, and the duration of the occult, reactors a, and reactors b stages are represented by λ_1, λ_{2a}, λ_{2b}. The equations for the probability of testing positive to the control strategies included sensitivity (Se), specificity (Sp), and correlation coefficients between negative ($\rho\Delta$) and positive ($\rho\Delta_S$) results, respectively.

Figure 2.

Median, 5th, and 95$^{\text{th}}$ percentile estimates of bTB-prevalence per month simulated for the model output of 500 iterations in a 500-size herd, with the application of *status quo*(Skin_series) and six alternative strategies. The red vertical line indicates when 50% of the simulations reached bTB-eradication for each strategy, and the shadow shows the range of months in which eradication is reached for 90% of the iterations.

Figure 3.

Median, and 95$^{\text{th}}$ percentile estimates of bTB-prevalence simulated for the model output of 500 iterations in a 500-size herd, with the application of *status quo* (Skin_series) and six alternative strategies. Simulated

estimates for bTB-prevalence in adults, representing 75% of the herd population (pink), and estimates for calves representing 25% of the population (turquoise) are shown per month.

Figure 4.

Median and 95_{th} percentile of the proportion of animals testing positive (pink), and testing true positive (turquoise) the*status quo* (0.Skin_series) and the six alternative control scenario per month.

Appendix: Supplementary material

Figure S1.

Figure S2.

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