EVALUATION OF CONTROL STRATEGIES FOR ERADICATION OF BOVINE TUBERCULOSIS IN ENDEMIC SETTINGS

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Research abstract

Bovine tuberculosis (bTB) is an ancient, zoonotic, infectious disease of cattle that has an important impact in animal and public health. Within the United Nations Sustainable Development Goal 3 to "ensure healthy lives and promote wellbeing," the WHO pursues by 2030 "the end of the human tuberculosis epidemic." To achieve this goal the burden of zoonotic-bTB needs to be abolished globally. In spite of many efforts and resources invested in its eradication, bTB is still endemic in many countries.

The intradermal tuberculin test and the slaughter of bTB positive animals, with the slaughter surveillance, are the basis of most of the bTB-control and eradication programs in place. However, the accuracy of intradermal testing tends to vary broadly with factors inherent to the settings (country) in which the test is carried out (resources, training, personnel, climate, and animal population), and related to the individual immunity of the animal (such as anergic periods or cross-reactivity with other *Mycobacterial* infections).

In Uruguay, in spite of many efforts dedicated to bTB eradication, this disease has reached unprecedented prevalence levels in large, intensified dairy systems in the past years (2010-2018). Trends of dairy consolidation, characterized by a steady decline in the number of dairy farms, increases in herd-size and rearing intensification, were associated with this bTB-prevalence growth in dairy systems. This raised concerns regarding the suitability of the bTB-control strategies to reach eradication in this evolving demographic and management scenarios.

The overarching goal of this dissertation was the assessment of current and alternative control strategies for bovine tuberculosis in high-prevalence endemic settings, considering the identified demographic and management risk factors, to guide the design and implementation of optimal control and eradication procedures through mathematical modeling. In order to achieve this goal, we initially used Bayesian statistical approaches to identify to the limitations of the current testing protocol under field conditions and assessed alternative diagnostic tools, which could help to constrain those limitations. Specific aims fulfilled and the main findings of this research were:

a) The characterization of the association between bTB diagnostic results and those obtained in Johne's disease (JD) (another important and widespread mycobacterial infection in cattle) diagnostics at the herd and individual level in an endemic high bTB-prevalence in which JD was present (scenario I), and individual association in high prevalence bTB- and JD-coinfected herds (scenario II). We demonstrated, in the scenario I, an association between the herd bTB status and the results obtained in a JD-ELISA. In addition, we determined, at the individual level an increased chance of positivity in the JD-ELISA when animals were frequently (>3 within a year) and recently (within 90 days) inoculated with intradermal tuberculin. For scenario II, we characterized the association between bTB- and JD-diagnostic result at the individual level in two farms in which both diseases were present at high prevalence. We observed a higher frequency of bTB-positive animals in the JD-positive population, with a significantly lower agreement between the caudal and cervical comparative intradermal tests compared to the JD-negative population.

b) The evaluation of the accuracy in herds heavily bTB- and JD- coinfected of two in-vitro (interferon-gamma release assay–IGRA- and ELISA) never previously used in Uruguay with the demographic and management risk characteristics identified (large herds, with a frequent and large number of animals, moved, and intensified). We determined the posterior estimates for sensitivity (Se) and specificity (Sp) from latent class models for IGRA and ELISA. We evidenced that IGRA was as sensitive (75-78%) as the intradermal tuberculin caudal fold test (CFT), and more sensitive than the serial use of CFT and intradermal comparative cervical test (CCT). Also, its specificity (90-96%) was superior to the one of the CFT and equivalent to the use of CFT-CCT. Estimates for the performance of the ELISA reached limited Se (~52%) and good Sp (~92%).

c) The assessment of bTB-within-herd dynamics and the epidemiological and performance effectiveness (uninfected false positive unnecessary slaughters) of six alternative control strategies involving single and parallel combinations of different tests (IGRAs, CFT, CFT+IGRA, CFT+ELISA, IGRA+ELISA) in large herds highly co-infected with bTB and JD. We concluded that any of the six alternative strategies assessed improved the time to bTB-control, as determined by the time-to eradication and time-to-regain the officially tuberculosis-free status, or reduce the false positive slaughter rate overall comparing to the status quo strategy (CFT-CCT in series). We characterized the role of the young cattle category (<12 months) on maintaining bTB-infection for longer periods when applying the alternative strategies to the status quo in highly bTB- and JD- coinfected dairy herds in Uruguay. We showed the importance of targeting

control strategies to younger animal categories, the potential benefit of using the IGRA in the initial stages of the control when bTB-prevalence is high without incurring in additional unnecessarily slaughters, and the poor expectations of the use of ELISA in parallel combination with CFT or IGRA.

Overall, we demonstrate that JD has an effect in bTB-diagnostic results at the herd and individual level in high prevalence bTB and JD coinfected populations studied, which needs to be addressed in the planning of bTB-control programs, specifically in regards of the performance of the bTB-diagnostic tools used. Still, in this co-infected scenario, the use of IGRA notably improved the sensitivity of detection in these herds, which can be beneficial in declining initial high bTB-prevalence levels. However, it is crucial to incorporate bTB-testing in young animals (<12 months) to break disease transmission and achieve prompt eradication.

With the assessment of bTB-control strategies in high prevalent endemic areas, incorporating the effect of JD-coinfection in the test performance, and herd risk factors associated with bTB (size, movements, intensification), represents the first attempt to integrate field risk factors for the diagnosis of bTB, and JD-coinfection in the design of control strategies for heavily infected herds.

Further studies would be required in order to determine the best bTB-control strategy resulting from the interaction between bTB- and JD- epidemiology, test performance, and economic costs, while acknowledging the country logistics and socio-cultural perceptions. Nevertheless, this research contributed to enhancing the understanding of

bTB-patterns in heavily endemic populations. The use of the diagnostic and modeling tools presented here can be the foundation of optimal bTB-control strategies to reach eradication when depopulation is not suitable.

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CHAPTER 1 – Introduction

1.1 Chapter summary

Bovine tuberculosis (bTB) is an ancient, zoonotic, and infectious disease of cattle that has a significant impact in animal and public health. This chapter briefly describes the general characteristics of bTB burden, epidemiology, and diagnostic tools that will be essential for the understanding of this dissertation. It also introduces Uruguay, the bTB-endemic country that motivated this dissertation, including its cattle production system characteristics, bTB-epidemiology, surveillance, control, and eradication program and its limitations. Finally, we identify the gaps and limitations in the current bTB-control program in this endemic setting and the goals of this dissertation.

1.2 General characterization

Etiology, hosts, transmission, clinical signs, and pathogenesis

Bovine tuberculosis (bTB) is an ancient, zoonotic, and infectious disease of cattle that has an important impact in animal and public health. It is caused by members of the *Mycobacterium tuberculosis* complex (MTBC) (Braun & Lebek, 1958; McMurray, 1941; Schmiedel, 1968). *Mycobacterium bovis* (*M. bovis*) is the main MTBC member causing disease in cattle, which origin dates from more than 10,000 years ago in association with domestication of cattle and early farming (Good, Bakker, Duignan, & Collins, 2018); however in recent years *Mycobacterium caprae* (*M. caprae*) has been described as another important agent affecting cattle in central and eastern Europe (Alicia Aranaz, Cousins, Mateos, & Dominguez, 2003; Muller et al., 2013; Rodriguez et al., 2009; Zanardi et al., 2013).

Transmission in cattle occurs mainly when a susceptible animal inhales an infective dose of aerosolized *M. bovis*, from a bTB-infected animal (Neill et al., 2001; Neill et al., 1994; Phillips et al., 2003) , or when sucking calves ingest contaminated colostrum or milk (Domingo, Vidal, & Marco, 2014; F. D. Menzies & Neill, 2000). Although *M. bovis* is an obligate pathogen, its bacterial structure allows it to survive in soil, water, and vegetation for 2 years when conditions are optimal (Morris, Pfeiffer, & Jackson, 1994) making ingestion of contaminated water or feed another possible transmission route in cattle, and the most likely cause of spillover to wildlife (Fitzgerald & Kaneene, 2013). Additionally, but less frequently reported, bTB-transmission paths can involve venereal, trans-placental, intra-mammary, and cutaneous route (Fraser D.

Menzies, Abernethy, Stringer, Honhold, & Gordon, 2012; Phillips et al., 2003; Vural & Tunca, 2001).

A bTB-infected animal is hardly identifiable by the clinical signs until the advanced stages of the disease when the most characteristic symptoms are wasting, debilitation, emaciation, and loss in production and body condition. When the animal is infected by *M. bovis* it develops an immune response that, if it does not eliminate the pathogen, develops a granuloma, which is generated by the accumulation of macrophages, lymphocytes, and dendritic cells in the primary site of infection (Cassidy et al., 1998), the so-called tubercle. This tubercle can stay latent, or reactivate as a result of a rupture, second infection, or deprivation of the immune response (e.g., stress) causing bacterial dissemination and subsequent infection of other organs (Domingo et al., 2014). The tubercles may be visible as early as three weeks after infection (Cassidy et al., 1998), and might be associated with clinical signs (Thoen, Karlson, & Himes, 1981). Between 9 and 20% of the infected animals might shed the *M. bovis* through the respiratory system, secretions, feces, milk, or urine for up to 38 weeks (Neill, Hanna, O'Brien, & McCracken, 1988; Neill et al., 1994) , becoming the main source of transmission to the other animals.

While cattle is the main host of *M. bovis*, infection has been reported in a wide range of domestic mammals, such as sheep (Muñoz Mendoza et al., 2012) , swine (Barandiaran, Martínez Vivot, Pérez, Cataldi, & Zumárraga, 2015; Jenkins et al., 2011), goats (Aranaz, 1999), and equines (Keck, Dutruel, Smyej, Nodet, & Boschiroli, 2010; Sarradell et al., 2015) , and wild animals, such as buffalo, antelope (de Garine-Wichatitsky et al., 2010; Shury, Nishi, Elkin, & Wobeser, 2015), deer (Miller &

Sweeney, 2013; Wobeser, 2009) , badgers (Buzdugan, Chambers, Delahay, & Drewe, 2016; Byrne et al., 2015) , wild boars (Alicia Aranaz et al., 2004; Gortazar et al., 2011) , and possums (Lisle et al., 2005; Nugent et al., 2015), which may contributes to disease epidemiology.

Given, these domestic species are in close contact with human populations, bTB is an important zoonosis (Cosivi et al., 1998). Similar to cattle, in humans, bTB is most often transmitted by aerosol exposure to the bacilli, or by the ingestion of unpasteurized milk, or contaminated food (Müller et al., 2013; Rua-Domenech, 2006).

Disease burden

Within the United Nations Sustainable Development Goal (SDG) 3 "Ensure healthy lives and promote wellbeing," the end-TB strategy pursues by 2030 "the end of human tuberculosis epidemic". In 2016, the World Health Organization estimated 147,000 new cases of zoonotic-bTB globally, with ~10% of deaths due to the disease, in which it's presumed that African and Southeast Asian countries had the heaviest burden (WHO). However, the true burden is likely underestimated, given estimates are mainly provided by countries in which bTB-programs are in place, and no reports are available from those without control or surveillance strategies in place (Olea-Popelka et al., 2017). The cultural and social traditions of some developing countries in which the close interaction with animals and the consumption of raw milk and animal products is a frequent practice make control difficult and likely increases zoonotic-bTB incidence.

Also, from the 179 countries in 2016, that reported their animal health status to the OIE (World Organization for Animal Health), ~50% declared the presence of bTB in

their animal populations. This indicates the global distribution and persistence of the disease in spite of efforts imposed in their control in many developed and developing countries (Bezos et al., 2014; de Kantor & Ritacco, 1994; Morris, 2015; Palmer & Waters, 2011; Picasso et al., 2017; Robinson, 2015), which raises the complexity in achieving the United Nations SDG-3.

The zoonotic dimension was the initial incentive to pursue the eradication of bTB and implement national programs mostly based on test-and-slaughter of infected animals (Pfeiffer, 2013). Furthermore, the costs associated with the disease presence, losses in animal production (milk, meat), welfare, early culling, and limitations in the trade of animals or animals products furthermore motivated stakeholders to seek the eradication of bTB from their herds. In countries with an established bTB-surveillance and control program, the most of the economic burden of the disease is linked to its implementation (Gormley, Anderson, & Nugent, 2017; Pfeiffer, 2013; Tschopp et al., 2013).

1.2 Immune response, antemortem and ancillary diagnostic tools

The acquired immune response triggered by the infection of *M. bovis* involves two immunity types which develop after an initial interaction with the pathogen (Kindt, Osborne, & Goldsby, 2007), first, the cell-mediated (CMI) and secondary, the antibody-mediated (AMI). The CMI is the most prominent and important response in the first stages of the disease and mainly relies on lymphocytes T (Baldwin & Telfer, 2015). The lymphocytes T helper 1 (Th1) are the ones that have a greater influence in the bTB-CMI, inducing the production of gamma-interferon (IFN-γ), and other cytokines (e.g. IL-17) responsible for the delayed-hypersensitivity type IV (Robinson, Orme, & Cooper, 2015)

that are the main target of most of the diagnostic tests. Lymphocytes T helper 2 (Th2) are involved in the development of the humoral response that will play a role in the more advanced stages of disease (Pollock, Welsh, & McNair, 2005). The emergence of the humoral response (AMI) is typically associated with the fade (or anergy) of the CMI (Mcnair et al., 2001) (figure 1.5.1). Until now, the period in which is possible to detect the immune response against bTB is not completely defined. Different studies reported the detection of CMI as early as 14 days until 119 days in natural and experimentally infected animals (Kao R. R., Roberts M. G., & Ryan T. J., 1997; Neill, O'Brien, & Hanna, 1991; Perez, Ward, & Ritacco, 2002; Pollock et al., 2001). Still, the median initial time to detection is 41 days (Alvarez, Bezos, et al., 2014).

Most of the animal health authorities base their national bTB-surveillance and control programs on the prompt detection and slaughter of infected animals to avoid transmission of the disease. The most used antemortem diagnostic methods are based on the detection of the CMI (OIE). The single or comparative intradermal tuberculin test (SIT, CCT), detecting the delayed-hypersensitivity type IV (Monaghan et al., 1994), and the interferon-gamma release assay (IGRA), detecting the gamma-interferon produced by lymphocytes T (Wood, Corner, & Plackett, 1990; Rothel et al., 1992).

Intradermal tuberculin test

In spite of the long time since its first use (>100 years) (Good et al., 2018) the intradermal tuberculin test is still the bTB diagnostic test of election for cattle. The different variations of the tests intended to identify the delayed-hypersensitivity reaction triggered by the production of cytokines by the lymphocytes Th1 after challenge by the

inoculation of a purified protein derivate from the *M. bovis* AN5 strain (PPDb) (Monaghan et al., 1994). If the animal was previously exposed to *M. bovis,* it develops a local hypersensitivity reaction in the inoculation site, which is maximum after 72 (± 4) hours of inoculation. The single intradermal test (SIT) has two variants currently used worldwide in terms of the inoculation site, with different performance (sensitivity and specificity) (Bezos, Casal, et al., 2014). In Europe, the intradermal inoculation is performed in the neck (single cervical test –SCT-) (Council Directive 64/632/EEC, 1964), and in Latin-America (de Kantor & Ritacco, 1994),North- America and New Zealand in the tail (caudal fold test–CFT-) (USDA/ APHIS 91-45-011). Any detectable reaction that increases the thickness of the skin at the inoculation site (>2 or 4 mm) and/or presence of local clinical signs (inflammation, oedema, pain, exudation and/or necrosis) is considered as a positive reaction (Monaghan et al., 1994).

One of the main limitations associated with the SIT is the potential occurrence of cross-reactions due to previous sensitization with other environmental or pathogenic mycobacteria(Gilot & Cocito, 1993). To overcome this limitation and improve the performance of the SIT, a second intradermal test, the comparative cervical intradermal test (CCT) can be performed, consisting in the additional inoculation of a PPD from *M. avium* (PPDa) next (12.5 cm apart) to the PPDb inoculation site. When the difference in skinfold thickness in the PPDb inoculation site is ≥4 mm than the PPDa animals are categorized as reactors (Monaghan et al., 1994).

Interferon-gamma release assay (IGRA)

The IGRA was developed ~30 years ago in Australia, and in the past years has been recommended as an ancillary in-vitro diagnostic assay for the intradermal test (OIE, 2009). The IGRA is performed in two steps. First, the heparinized whole blood drawn from the animal is incubated with the PPDb (or different peptides derived from *M. bovis*), PPDa, and control buffers (PBS) to stimulate the IFN-γ release by sensitized lymphocyte T to the different antigens; and secondly, an enzyme sandwich immunosorbent assay is used to harvest the IFN- γ released in the plasma by the T-cells. Results obtained from the optical density (OD) read are used to classify the animals as positive or negative.

Enzyme-linked immunosorbent assay (ELISA) and other serodiagnosis tools

The use of diagnostic tools targeting the bTB-AMI detect animals, which likely are transmitting and shedding the pathogen (Pollock et al., 2001; Ritacco et al., 1991; I. Schiller et al., 2010). For that reason, their use of bTB-control programs is not officially recommended (OIE, 2009). However, can be useful to detect animals in the anergic stage (Casal et al., 2014; Pollock et al., 2005).

Diverse serological diagnostic tools have been described for bTB diagnosis, but the most widely used is the ELISA with the MPB83 and MPB70 antigens (Waters et al., 2011; Whelan et al., 2008). The ELISA technique is based on the capture of the specific antibodies with an antigen previously bound to an enzyme able to produce a quantifiable reaction (e.g., color, temperature) (Cho et al., 2007).

Other serological tests have been developed to improve the ability to detect the AMI, such as the multi-antigen print immunoassay (MAPIA) (K.P. Lyashchenko et al.,

2008), lateral-flow rapid test (LF), or the double-recognition ELISA (DR-ELISA) (Bezos, Casal, et al., 2014), but, their use is not frequent.

Other ancillary diagnostic tests

Post mortem diagnosis has been an essential component of bTB-passive surveillance in every country with a national bTB-program. The identification of macroscopic lesions (tubercles) during the examination of the carcass of animals, is essential. However, it requires that the animal has visible lesions in the lymph nodes to reach a good sensitivity (Diana L. Whipple, Bolin, & Miller, 1996).

M. bovis culture is still considered the "gold standard" for bTB (OIE, 2009). The ability to correctly identify infected animals can be improved when the tissues cultured include bTB macroscopic lesions. However, because of the slow metabolism of *M. bovis*, it can take up to three months to obtain a positive result. Is in these scenarios in which the polymerase chain reaction, for detection of specific DNA, offers a potentially faster and more flexible option (de la Rua-Domenech et al., 2006). However, this technique is still not broadly used because is reported to be less sensitive than the culture (Gormley et al., 2014).

Performance of diagnostic tests used for bTB-control

To assess and compare the performance of a diagnostic test it is necessary to determine its sensitivity and specificity. The probability of testing positive given the animal is bTB-infected represents the sensitivity (Se), and the probability of testing negative given the animal is non-infected represents the specificity (Sp) (Dohoo, Martin, & Stryhn, 2009). The accuracy (performance) of the previously described bTB-diagnostic

tools has been extensively studied mainly in North American and European countries, yielding an important variability in the performance achieved (Bezos, Casal, et al., 2014; de la Rua-Domenech et al., 2006; Nuñez-Garcia et al., 2018).

The accuracy of the intradermal tuberculin test may differ depending on the type and location of the inoculation. Estimates for the CFT (Farnham, Norby, Goldsmith, & Wells, 2012a) and the SCT (Bezos, Casal, et al., 2014) were reviewed independently. Traditionally, a slightly improved sensitivity (Se) was attributed to the SCT (Se: 80.2-100%) over the CFT (Se: 80.3-93%). Still, field studies reported lower Se estimates of 53% (27.3–81.5, 95% CI) and 69.4% (40.1–92.2, 95% CI), respectively, depending on the interpretation criteria used (Alvarez et al., 2012). In addition, as previously mentioned, the Sp of the SIT is not perfect, with estimates between 89.2 and 95.2% (CFT), and 55.1 to 99% (SCT) (Bezos, Casal, et al., 2014). The accuracy of the CCT when applied as a confirmation test after the SIT with the objective of improving the specificity of the bTB-diagnostic, included a Se of 70 and 89.9% (depending on which SIT was performed first) and a Sp of 78.8 to 100% (Bezos, Casal, et al., 2014; de la Rua-Domenech et al., 2006) .

The use of IGRA (with various antigens) as an ancillary diagnostic tool in many bTB-control programs worldwide is generally intended for the improvement of the Se of detection. The performance of the IGRA after the intradermal inoculation of PPDb may improve in terms of Se due to an anamnestic/booster effect (Palmer et al., 2006). Recently a meta-analysis reported Se estimates in the range of 49 and 90% depending on the antigen used. Although the Sp of 96.6 (85-99.6, 95% CI) tend to be lower than the intradermal test, the use of more specific peptides improved the initially reported

performance (99-100%) (Aagaard et al., 2006; Nuñez-Garcia et al., 2018; Vordermeier et al., 2001).

While not part of any official bTB-control program yet, the ELISA has potential when strategically used with the intradermal test (Casal et al., 2014). The performance of the ELISA has not been broadly explored but estimates of 33-84%, and 81-100% for Se and Sp respectively have been reported (Al-Mouqatea et al., 2018; Casal et al., 2014; Nuñez-Garcia et al., 2018).

1.3 bTB-endemic setting

Uruguay: cattle production and bTB-epidemiology

Uruguay is a country located in the southeastern coast of the South American continent (Figure 1.5.2). The vast majority of its land extension (175.020 sq. km) is utilized for agriculture production, which accounts for ~8% of the gross domestic product (GDP) of the country. In a country of >18 million heads of livestock, activities related to its production represents >50% of the agro-industrial GDP (DIEA, 2018) and relates to 60% of the employment within this sector (Riella & Ramirez, 2012).

The dairy industry in Uruguay accounts for ~10% of the cattle farms and is mainly located in the western and southeastern areas of the country. Uruguayan dairies historically had small-to-medium size herds (<360 animals), and semi-pastured based production with low rearing intensity. However, in the past decade the trends of consolidation, characterized by a steady decline in the number of dairy herds with an attrition rate of 15% (2011-2018) characterized by exiting small herds and increased consolidation. The dairy industry in Uruguay became a particular example of the

continuous evolution of the animal production systems in the country and the need to adjust and redesign animal health programs accordingly.

Historically, with the use of the current bTB-control program, the prevalence of bTB consistently decreased to 1% in 1990, and <0.001% in 2010 according to official records (DSA-MGAP, 2016), showing a trending potential to achieve eradication.

While the bTB-prevalence in Uruguay did not vary substantially, in the past years (2011-2018) there was an increase in the number of bTB-outbreaks in dairy herds (figure 1.5.3), with a within-herd median prevalence >10 times higher than the reported for the previous similar period (2003-2010) (Animal Health Division, MGAP, 2017).

Recent epidemiological assessments revealed the spatial and animal movement network clustering of bTB-outbreaks, with a spatial aggregation of cases in the western areas of the country at the time that the number of bTB-outbreaks started to rise (2011-2013). Herd-level risk factors analysis concluded that larger dairy herds (>360 animals) had a 14 times higher risk of bTB-breakdown, and certain management practices, such as large sizes-batches of incoming animals (>44), led to twice the risk than those purchasing fewer animals (Picasso et al., 2017). A deeper analysis of movement patterns within the cattle population in Uruguay showed that the majority of farms had few to no contacts, whereas the 10% most highly connected farms accounted for 72-83% of animals moved annually. Dairy premises were responsible for the majority of the outward movements in the network, with 75% of them connected to at least one other farm by out-shipments (VanderWaal et al., 2016) (Figure 1.5.4). The high dairy interconnection highlighted the risk for these farms to contract bTB and spread the disease.

bTB-surveillance and control program

bTB-active surveillance program in Uruguay was designed to target the most common risk factors associated with bTB (Humblet, Boschiroli, & Saegerman, 2009), which were typically associated with the dairy systems. Dairy animals are often maintained in the herd for several years, with management practices that involve frequent commingling of animals (milking routines), and feeding habits that could increase the risk of inhalation of aerosolized *M. bovis,* together with low biosecurity and excess of manure accumulations that can increase the risk for the fecal-oral transmission. Passive bTB-surveillance was implemented at the slaughter of animals intending to detect potential bTB circulating in the beef industry.

The current regulations for bTB-surveillance- and-control are based on serial testing -and slaughter of reactors in the dairies, and the visual examination of the carcasses for detection of any tubercle-like lesion at slaughter with the use of culture as an ancillary diagnostic test for bTB-confirmation (MGAP, 1989). The status of Officially Tuberculosis Free (OTF) herd in dairies is obtained when annual testing is negative for the complete herd, and no evidence of bTB-lesions are found at slaughter; while beef herds are OTF when no macroscopic lesions are found at the slaughter of culled cattle.

In dairies, adult animals (>12 months old), are annually subjected to bTB- testing with the use of the CFT version of the intradermal tuberculin test. Animals with local clinical signs (inflammation, edema, pain, exudation and/or necrosis) or skin thickness increase > 4mm after 72 hrs post inoculation are reactors, and they are retested for confirmation, in a period <10 days or >60days, with the comparative cervical tuberculin

(CCT). When the difference in skin thickness between the two inoculations performed in the CCTusing *M. bovis* (PPDb) and *M. avium* (PPDa) antigen is >4mm, animals are considered positive and sent to slaughter within the month of detection (MGAP, 1989). Movements of animals from the herd are suspended until OTF status is regained. Slaughter procedures are followed under the official veterinary supervision and tissues from the carcasses must be submitted to the laboratory for *M. bovis* culture. After an infected herd is detected, a complete epidemiological investigation starts, and control strategies are applied, including the retesting of all eligible animals in the infected herd and contact herds (neighbors and connected by movements within the past two years) to disclosure evidence of bTB or exposure in those contact herds too. When the outbreak herd accomplishes two consecutive whole-herd negative testing results in a period no less than 60 to 120 days, it regains the OTF status.

1.4. Gaps and limitations of the bTB-control program in endemic settings

Generally, the accuracy of intradermal testing tends to vary according to several factors inherent to the situation in which the test is carried (resources, training, personnel, climate, and animal population), and related to the individual immunity of the animal (de la Rua-Domenech et al., 2006). Factors associated with the implementation setting can be summarized in a) factors inherent to the test (antigens or sample), and b) operating characteristics.

The factors associated with the implementation settings that are inherent to the intradermal test and can affect its performance relate to the potency of the tuberculin used, for instance: the improper manufacturing of the antigens, such as contamination

(purity), miscalibration of the potency, strain used; or the improper preservation of the antigen, such as time (expiration), temperature, light and humidity during storing (de la Rua-Domenech et al., 2006; Monaghan et al., 1994). Performance limitations associated with the operating characteristics include factors such as the dose inoculated in the animal, the location (subcutaneous instead of intradermal), inadequate time between inoculation and reading the results, misidentification of the animals or the PPD used (in the case of the CCT), errors in records between inoculation and reading of reaction.

Factors related to the animal immune system that impair the accuracy (Se or Sp) of the intradermal test can be associated with: a) management practices, b) physiological events, c) pathological events(Alvarez et al., 2014; Humblet et al., 2009). Different regular management practices can suppress the response of the immune system to the tuberculin inoculation, such as the administration of immunosuppressive drugs (e.g. corticoids), the repeated inoculation of tuberculins between 10-60 days after the previous inoculation, and the vaccination against other mycobacterial diseases (Johne's disease). Physiological events affecting the hypersensitivity type IV response are pregnancy, or stress situations, such as animal movements or nutritional deficits. Finally, animals with recent bTB-infections (<42 days), or in too advanced stages of the disease may fall into a non-reactive period in which the immune system will fail to react to the tuberculin inoculation. Together, false reactions (cross-reactions or suppression) can occur when animals are co-infected with other viruses (e.g., bovine viral diarrhea) or other mycobacteria (i.e., *Mycobacterium avium* subsp. *paratuberculosis* –the etiological agent of JD- or environmental mycobacteria) (de la Rua-Domenech et al., 2006; Monaghan et al., 1994; Snider, 1982).

Although sometimes challenging, limitations associated with the implementing settings tend to be relatively easy to identify, correct, and mitigate by the use of good management practices. Similarly, immunological factors associated with management and physiological events can be mitigated when using a bTB-testing protocol. Nevertheless, pathological events associated with the immune system of the animal tend to be highly variable and need to be studied and addressed through the integrated and strategic use of the available diagnostic tools.

For Uruguay, an in-silico assessment of the performance of the program using an integrated within- and between-herd model parameterized for the bTB-endemic Uruguay cattle population indicated that slaughter sensitivity had little impact on the overall surveillance sensitivity when combined with skin testing. At the same time, the model suggested that the sensitivity of the surveillance strategies (i.e. CFT and CCT in series) was limited (0.53, 95th 0.46–0.62), and if testing efforts were relaxed (risk-targeted surveillance), prevalence estimates did not vary significantly (VanderWaal et al., 2017). This results highlighted the need for improvement of sensitivity of the field tests to detect bTB-infected animals.

The current scenario in Uruguay, characterized by **a)** an increase of bTB-dairy outbreaks with a high within-herd prevalence, **b)** the evolution of the dairy into a highly intensified system, and **c)** the limited accuracy of the bTB-testing scheme, raised the question of whether the current control strategies are suitable to achieve bTB-eradication in this (and similar) endemic settings.

In some countries, depopulation is a feasible alternative to eradicate bTB from high prevalence herds (More, Radunz, & Glanville, 2015). However, the complexity of its implementation, cost, and social impact (Giovanna Ciaravino et al., 2017) that slaughtering a complete herd imposes, makes this strategy unfeasible in Uruguay.

The gaps in the understanding of the performance of different diagnostic strategies accounting for field-test accuracy, cattle demographic characteristics, and JD coinfection, when depopulation is not feasible, needs to be addressed. The Uruguayan current bTB-epidemiology and production characteristics need to be studied to approach this gap.

Testing empirically in vivo different scenarios for the control of bTB, it would be unmanageable and sometimes unethical to incur in its costs and risks. Is in this situation in which mathematical models can help us understand the interactions of the underlying factors that affect disease transmission (de Jong, 1995), connecting theories, and testing the counterfactual hypothesis.

Mathematical transmission models were previously used to mimic bTB dynamics (Alvarez, Bezos, et al., 2014; G. Ciaravino et al., 2018; Kao R. R. et al., 1997; Perez et al., 2002; Rossi, Aubry, Dubé, & Smith, 2019), since they allow accounting for the bTB-chronic nature, with long and variable incubation periods, and biological variabilities. Moreover, bTB-models incorporate different characteristics of the production systems, while avoiding the risks and the costs of in-vivo implementation (Halasa & Dürr, 2017), which makes them a remarkable tool to contribute to this research.

1.5 Goal of the dissertation

With the purpose of filling the gap explained above, the overarching goal of this dissertation is to assess the usefulness of alternative diagnostic tools for bTB in high prevalence endemic areas in which depopulation is not feasible, to guide the design and implementation of optimal strategies to succeed in the eradication of bTB in endemic populations.

In order to achieve this goal, we specifically need to understand the potential immunological response to the current testing protocol under field conditions and assessment of tools that will help us to avoid those limitations. The diagnostic interaction with Johne's disease (JD) at the herd and individual level (chapter 2 and 3), and the performance of diagnostic tools that can improve detection during the non-reactive periods (early and late stages of infection) (chapter 4) will be essential to ultimately, identify strategies that might be the basis of control programs in similar endemic settings (chapter 5), (figure 1.5.5).

Specifically, in **chapter two**, a characterization of the association between bTB diagnostic results and results obtained from a JD-ELISA at the herd and individual level in an endemic high prevalence setting was performed. We selected the Castilla y Leon (CyL) autonomous community in Spain for this study because it has the largest cattle population of Spain, bTB-prevalence levels >1%, a test-and-slaughter bTB-control program in place, and JD present though at an unknown level in the cattle herds with no official control program in place, making it an optimum setting to address this goal.

Chapter three aimed to determine, characterize, and estimate the association between bTB- and JD-diagnostic results at the individual level in two farms in which both diseases were present at high prevalence. To understand this, we selected two chronically bTB-infected dairy herds in Uruguay, with high (>7%) apparent prevalence based on intradermal testing and with a history of JD-positive serological results.

In the **fourth chapter** of this thesis, we estimated the accuracy of two in-vitro assays never used previously in Uruguay in two herds heavily infected with the demographic risk factor previously described, and highly co-infected with JD, to understand their potential performance in those scenarios. The two commercial assays for in-vitro diagnosis of bTB selected were the IGRA and the ELISA, which had been used to improve the sensitivity of diagnostic in the non-reactive periods in which intradermal tests fail.

Finally, in **chapter five**, we evaluated alternative strategies to control and eradicate bTB from chronically- and heavily- infected herds based on the use of different combinations of diagnostic tests in JD-co infected setting using mathematical modeling.

As a whole, this dissertation explores some of the most critical factors affecting bTB-control and diagnosis and evaluate opportunities to mitigate them under field conditions in endemic populations. In a broader view, the diagnostic and modeling tools presented in this research can contribute to the foundation of the optimal bTB-control strategy in endemic settings, when depopulation is not suitable.

1.5 Figures

Figure 1: Schematic representation of the diversity of responses of the bovine immune system to various tests for bTB.

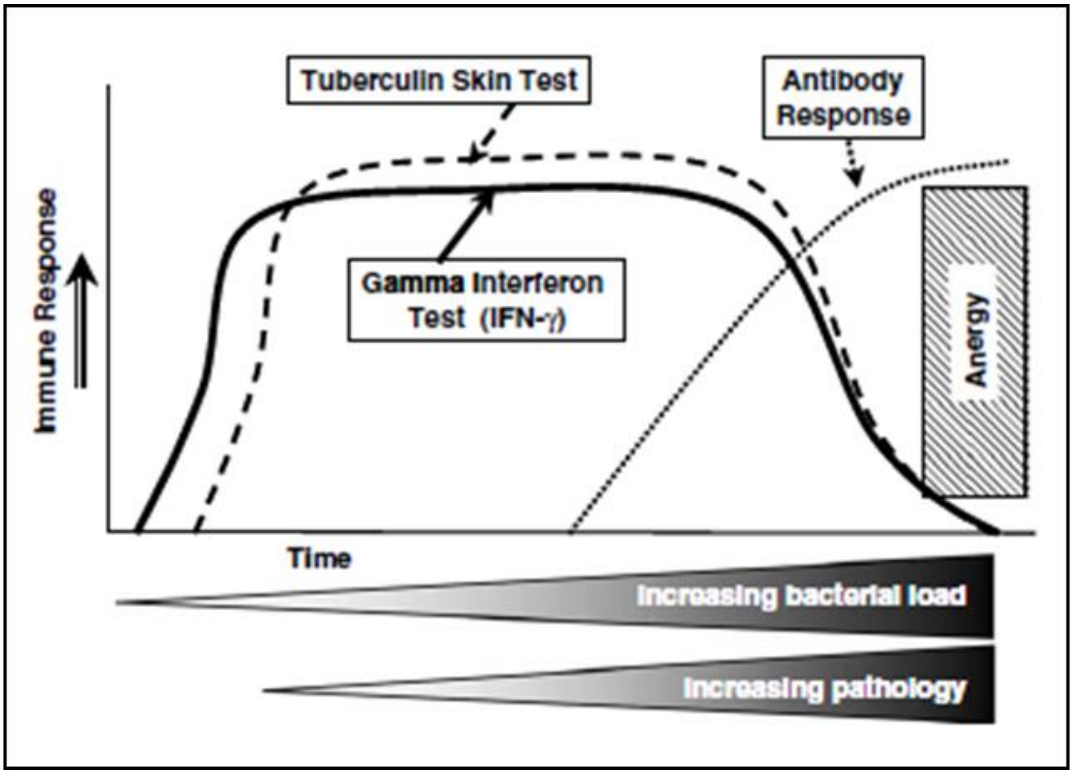


Figure credit to de la Rua-Domenech et al., 2006).

Figure 2: Location of Uruguay.

Figure 3: Bovine tuberculosis positive farms reported per year in Uruguay (2003-2018).

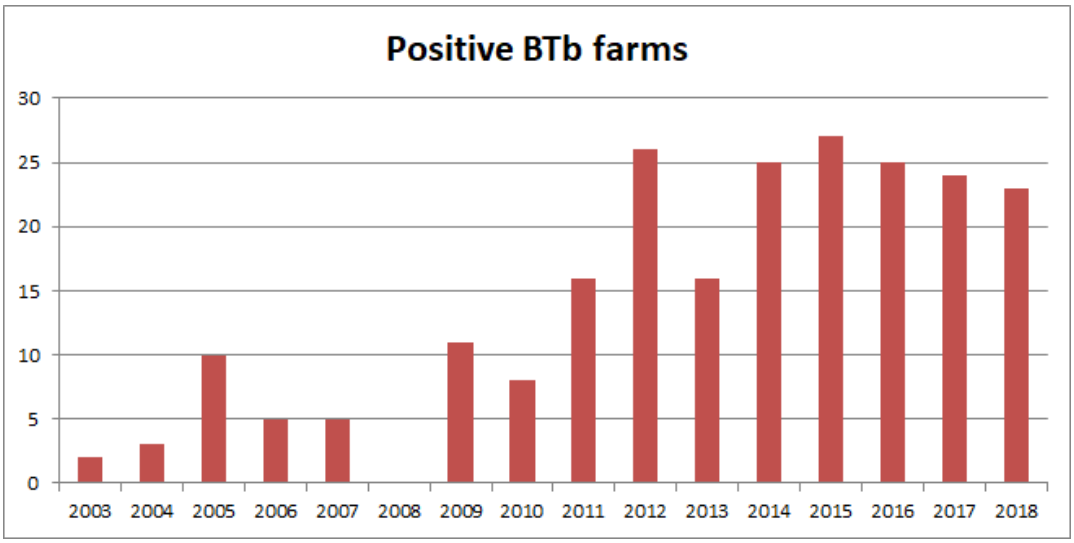
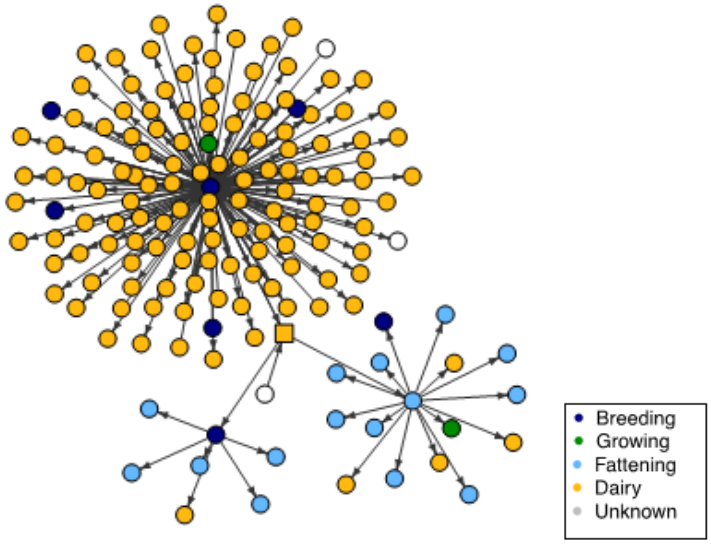
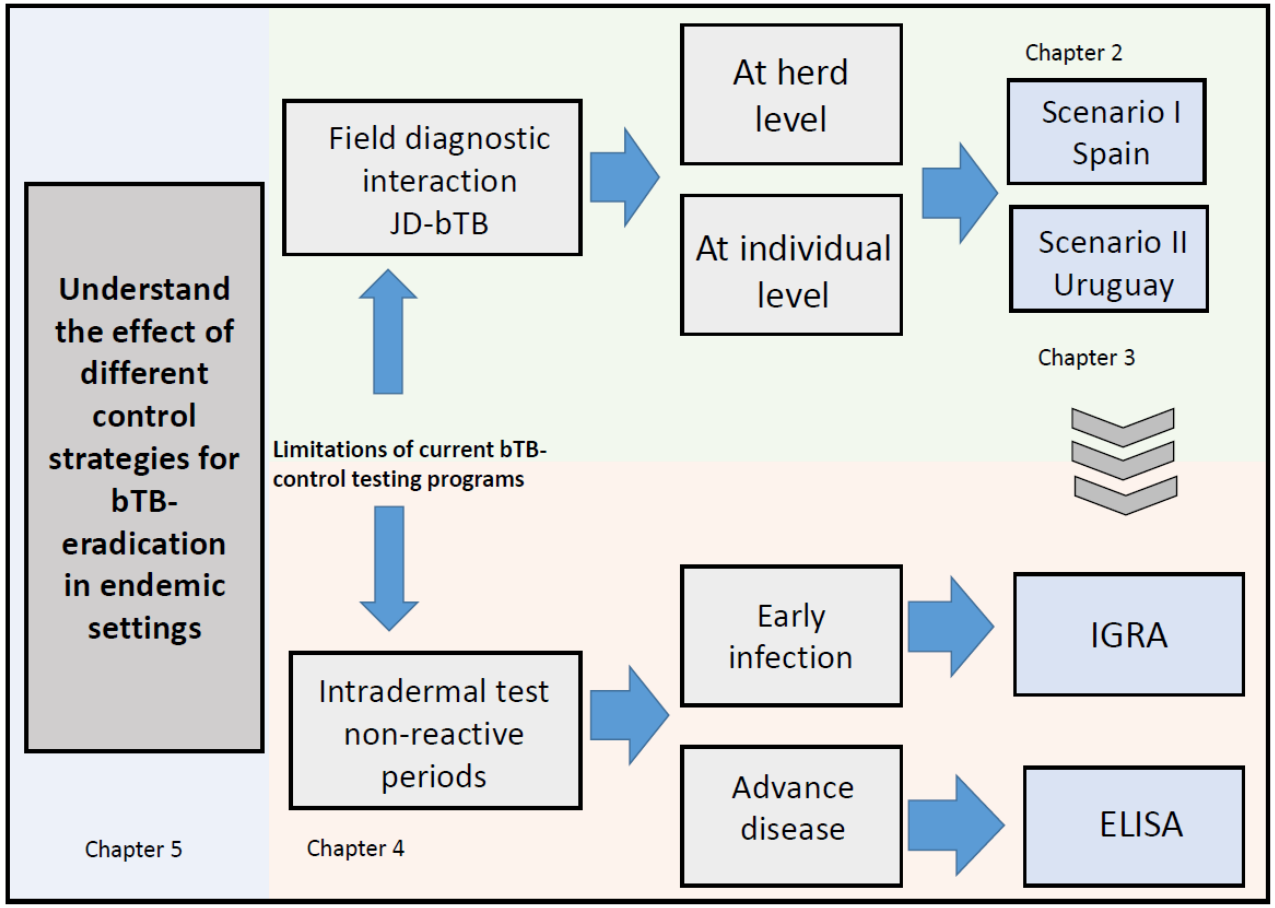


Figure 4: An example of the neighborhoods of a dairy farm in Uruguay.



Dairy focal node (yellow-square), drawn randomly from the farms with dairy production. These networks include all farms reachable within two steps, taking into account both the direction and temporal sequence of movements (reproduced from VanderWaal et al., 2016)

Figure 5: Schematic representation of the different specific aims and their interaction to address the overarching goal.



CHAPTER 2 – Association between results on diagnostic tests for bovine tuberculosis and Johne's disease in cattle

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2.1 Chapter summary

Bovine Tuberculosis (bTB) diagnosis is impaired by numerous factors including cross-reactivity with *Mycobacterium avium* subsp. *paratuberculosis*, which causes Johne's disease (JD). In addition, the effect of repeated bTB-intradermal testing on the performance of JD diagnostic tests is not fully understood. This chapter aimed to evaluate the impact of repeated bTB-intradermal tests under field conditions on the JD serological status of cattle. bTB-positive herds (n=264) from Castilla-y-Leon region in Spain were selected and matched with officially tuberculosis-free control herds. The association between JD- and bTB-status at the herd level was assessed using conditional logistic regression and, in herds with both JD- and bTB-positive animals; a Bayesian hierarchical mixed-effect model was used for individual-level analysis. A significantly higher risk of being JD-positive (OR: 1.48; 95%CI: 1.01 – 2.15) was found for bTB positive herds compared with controls. Individual results indicated that cattle tested >3 times/year, within the last 90 days and >12 months were more likely to be JD-positive. A skin-test related boost in antibody response could be the cause of an apparent increase in the sensitivity of the JD-absorbed-ELISA. Improved knowledge of the diagnostic interactions between bTB and JD facilitates the design of more effective control programs in coinfected herds.

2.2 Introduction:

Bovine Tuberculosis (bTB) is a chronic disease in bovines with a worldwide distribution causing a significant impact in both animal and public health (Muller et al., 2013; Olea-Popelka et al., 2017; "WHO | Tuberculosis," n.d.). Because of this zoonotic potential of its main causative agent, *Mycobacterium bovis*, measures for disease control and eradication in the livestock were first implemented in the 1890s in Denmark (Bang, 1908) and subsequently in most industrialized countries during the last century. All eradication programs are based on test-and-cull and depopulation policies (Bezos, Álvarez, et al., 2014; Pfeiffer, 2013). Detection of the disease at the herd level is an important part of the control programs. Therefore, the accuracy of the diagnostic tools being used is essential, since both false positive and false negatives will have severe implications for all stakeholders involved.

In Spain, the official bovine tuberculosis/bTB-free (OTF) status of the country has not been obtained yet, despite substantial efforts invested in the national bTB eradication program. However, the bTB herd prevalence in Spain has remained relatively stable over the past 15 years, ranging between 2.1% and 2.8% of the herds in 2000 and 2017, respectively. The herd prevalence varies significantly between regions (Ministerio de Agricultura y Pesca AyMA, 2017). The Castilla-y-Leon (CyL) autonomous community, in the northwest-central region of Spain, has the largest cattle population of the country. The bTB herd-level prevalence in CyL is >1% and, therefore, is classified as a high prevalence region according to the standards of the bTB eradication program of Spain. Approximately 30% of herds classified as bTB-

infected herds in 2015 were located in this region (Ministerio de Agricultura y Pesca AyMA, 2017).

As part of the eradication program, all herds are screened for bTB using the single intradermal tuberculin test (SIT). In Spain, the SIT assay is performed according to European regulations (Council Directive 64/432/EEC) and is applied in the cervical area of all cattle older than six weeks at least once every year. In addition, the interferon-gamma release assay (IGRA) is used in bTB-infected herds as an ancillary test in animals older than six months to maximize diagnostic sensitivity to detect all infected animals within the herd(Council Directive 64/432/EEC).

Failures to eradicate bTB have been attributed, at least in part, to the lack of accuracy of the bTB diagnostic tools (Irene Schiller et al., 2010). Many individual- and herd-level factors can impair the performance of bTB diagnostic assays, including cross-reactivity due to previous sensitization with other mycobacteria, resulting in both false negatives and false positives. For example, Mycobacterium *avium* subsp. *paratuberculosis* (MAP), the causative agent of Johne's Disease (JD), shares structural proteins and virulence factors with *M. bovis* and has been described as a frequent source of diagnostic interference when intradermal assay testing is used (Abdallah et al., 2007; Alicia Aranaz et al., 2006; Gilot & Cocito, 1993; Roussel, Fosgate, Manning, & Collins, 2007). However, the exact impact of JD on bTB diagnostic performance and, vice versa, the effect of bTB itself as well as the frequent use of the intradermal assay for the detection of bTB on the detection of JD, is still largely unknown. Some reports described an impaired sensitivity and/or specificity in at least one of the diagnostic tests (Alvarez et al., 2008, 2012; Alicia Aranaz et al.,

2006; Brito et al., 2014; A.E. Kennedy, Byrne, O'Mahony, & Sayers, 2017), while others reported a negligible effect (Dunn et al., 2005).

Johne's disease (JD), a chronic disease of ruminants with a worldwide distribution, is an important cause of the reduction in efficiency and profitability of the dairy industry (Over, Crandall, O'Bryan, & Ricke, 2011). Although the economic impact of the disease in the cattle industry varies depending on production systems (Barbara Dufour, Régis Pouillot, & Benoît Durand, 2004), it can be as high as US$ 22 to 200 per cow, as estimated for the United States, (Ott, Wells, & Wagner, 1999; Stott, Jones, Humphry, & Gunn, 2005). JD is also present in cattle herds in Spain, including CyL. However, at present little is known about its epidemiology and distribution in this region.

MAP-infected cattle initially develop a minor cellular response followed by the generation of antibodies in more advanced stages of the disease (S.S. Nielsen & Toft, 2008). This late antibody response is the target of a serum enzyme-linked immunoabsorbent assay (ELISA). Because of this late antibody response, the assay identifies only animals JD infected in an advanced stage of infection. Nevertheless, the ELISA is the most commonly used JD screening test because it has low logistic demands, it is affordable and is easy to implement, and the results are rapidly obtained (Scott et al., 2010). Interestingly, a possible effect has been described of the routine intradermal assay based on the inoculation of bovine and avian purified protein derivates of tuberculin (PPDs). The resulting effect is the so-called anamnestic rise of antibody levels, causing increased sensitivity of the ELISA, enhancing the detection

of JD-infected cattle (Aideen E Kennedy, O'Mahony, Byrne, MacSharry, & Sayers, 2017).

This chapter aimed to characterize the effect of the repeated use of bTB diagnostic tests on the JD status at the herd and animal level to evaluate the potential for diagnostic interference between these two mycobacterial diseases under field conditions. Specifically, we aimed at testing two hypotheses, namely, (a) frequent testing for bTB has an impact on the JD status at the herd level (as determined using a serological assay); and (b) there is an association between bTB and JD diagnostic results at the individual level in herds in which both diseases are present. Results presented here will help to understand, in quantitative terms, the relation between the two most important chronic diseases of livestock caused by mycobacteria, ultimately contributing to the evaluation and subsequent improvement of surveillance strategies in endemically infected countries.

2.3 Methods

Study population:

The study was conducted in CyL, located in the central area of Spain. Herds were selected using a case-control design. The history of bTB-infected herds present in 2015 was screened to select those that were already positive in 2013 and 2014 and had at least 10 animals ≥ 6 months of age (case herds). Each case herd was matched with a control herd (1:1) paired by known bTB risk factors such as production type (dairy, beef, bullfighting), herd size (<51, 51-100, 101-200 or >200 animals) and

geographic location (county) (Humblet et al., 2009). All control herds had been officially tuberculosis-free (OTF) for at least the previous 24 months.

Diagnostic tests:

All herds were routinely subjected to the official diagnostic tests mandatory in the bTB eradication program (the SIT test and, for known bTB-infected herds, the SIT in combination with the IGRA). Briefly, the SIT assay requires the intradermal inoculation of a bovine PPD (PPDb), prepared from a culture of *M. bovis,* in the cervical area and measuring the skin thickness at the inoculation site before the injection and after 72 hours. Applying the severe interpretation (according to Council Directive 64/432/EEC and R.D. 2611/1996): if an increase in skinfold thickness of >2mm or clinical signs (inflammation, oedema, pain, exudation and/or necrosis) were observed at the inoculation site, the animal was considered to be a bTB reactor. In known bTB positive herds, blood samples from all ≥6 month-old animals were also collected in tubes with heparin, transported to the laboratory and within 8 hours analyzed using the BOVIGAM ELISA (Thermo Fisher Scientific, USA) as described elsewhere (Alvarez et al., 2009).

Finally, cattle serum collected from the coccygeal vein of all animals from the selected case and control herds, obtained as part of the bovine brucellosis eradication program, were then screened using a JD serological test. JD specific antibodies were detected with a commercial Enzyme-Linked Immunosorbent Assay (ELISA). In this JD-ELISA, an ELISA plate coating a crude extract of MAP is used and involves an absorption step with a *Mycobacterium phlei* extract to reduce cross-reactivity with environmental mycobacteria, as described elsewhere (YOKOMIZO, YUGI, &

MERKAL, 1985). Animals with an S/P ratio ≥ 60% were considered positive as recommended by the kit manufacturer (Institute Pourquier, Montpellier, France). Herds were classified as JD-negative if all tested animals were seronegative and JD-positive otherwise. In addition, we assessed the effect of increasing the cut-off to classify a herd as JD positive to two animals to maximize herd-level specificity.

Data analyses

Association between bTB- and JD- diagnosis at the herd level

We explored the effect that the repeated use of SIT assay has on JD-ELISA results at the herd level in two ways. First, a univariable conditional logistic regression was used to estimate the strength of the association between the JD status and the bTB classification (case/control) of the herd; second, a Wilcoxon matched-pair signed rank test was applied to compare the number of JD positive animals in case versus control herds.

Association between bTB- and JD-diagnosis at the individual level

Herds, in which positive animals to both bTB and JD diagnostic tests were detected, were identified. In those herds, information on variables at the individual (age, time since the last SIT test, number of SIT tests undertaken in the previous year, and result in the concurrent SIT/IGRA) and herd (production type and herd size) levels hypothesized to be associated with the JD individual result was collected. Continuous variables were categorized into four categories based on previous knowledge or biological reasoning and data distribution (quartiles).

The association between the individual and herd variables and the response obtained in the JD ELISA at the individual level was assessed using a Bayesian multivariable logistic regression mixed model. A hierarchical model was used to account for the lack of independence between observations from animals in the same herd. Individual JD-ELISA results (positive/negative, JD_{ij}) from animal *i* in herd *j* were assumed to follow a Bernoulli distribution with probability p_{ij}, formulated as a function of the individual (β[n]) and herd (γ[k]) level fixed effects and the variability in risk given by the herd of origin as the random herd intercept (αj).

$$JD_{ij} \sim dbern(p_{ij})$$

$$\varphi_j = \alpha_j + \gamma_1 * z_{1[j]} + \gamma_2 * z_{2[j]}$$

$$\log(p[ij]) = \beta_0 + \beta_1 * x_{[ij]} + \cdots \beta_n * x_{[ij]} + \varphi_j$$

Herd-level variables ($z_{1,2}$) were included in the model as part of the herd-level random effect model (φ_j) and the best structure for φ_j was selected based on the lower deviance inference criterion (DIC) (D. J. Spiegelhalter, Best, Carlin, & van der Linde, 2002) of models that only included the intercept (β_0) at the individual effect. Then, individual-level variables ($\beta_{1...}$ β_n) were considered alternatively in univariable models and those in which a statistically important association (80% posterior probability intervals not including zero) was found were maintained in a multilevel multivariable model. Collinearity between covariables was assessed using a chi-square test.

Gaussian weakly informative priors [N(0, 100)] were used for the regression coefficients at the herd (γ) and individual level (β). The herd-level random intercept (α_j) was assigned a normal distribution in which the mean was parameterized using a normal distribution N(0,1), and the inverse of the variance was modeled as a Gamma(0.01, 0.01). The best-fitting model was selected based on lower DIC. Two-way interactions between covariates were assessed after partial pooling (also referred to as "recategorizing") the covariates as appropriate based on the posterior estimates results in following procedures described elsewhere (A Gelman & Hill, 2007). Briefly, each category of the covariates will have a posterior risk estimate; when the estimate was similar for two continuous categories, we pooled (or recategorized) them to assess its interaction with other covariates.

Models were fitted using OpenBUGS 3.2.2 (Thomas, O'Hara, Ligges, & Sturtz, 2006) via the R2OpenBUGS package (Sturtz, Ligges, & Gelman, 2005) from the R software (v3.2.4, R Foundation for Statistical Computing). Three independent chains were run for 7,500 iterations considering a burn-in period of 2,500 iterations. To eliminate potential autocorrelation in the posterior estimates, we selected one of every ten consecutive samples. Convergence was assessed in two ways: (a) graphically, by visually assessing the mixing of the three chains and (b) considering the Gelman-Rubin^R estimate, which compares the variability within and between the multiple simulated chains (Brooks & Gelman, 1998; Andrew Gelman & Rubin, 1992).

2.4 Results

Herd-level analysis

The majority (264/373, 70.8%) of the bTB-infected herds initially enrolled in the study were successfully matched with an OTF control-herd with similar characteristics (production type, size, and location), so the final sample size was composed of 528 herds and 56,131 animals tested for both bTB and JD. In the case herds, 120 and 1740 animals were positive to the SIT test and IGRA, respectively. Overall, 272 herds (147 cases and 125 controls) were classified as JD-positive (Table 1), with a median within-herd JD apparent prevalence of 2.34% (interquartile range (IQR): 1.12-4.44%) in the bTB-infected herds and 1.81% (IQR: 1.11-3.44%) in the controls. The univariate conditional logistic regression model suggested a significant (P=0.01) higher risk of being classified as JD- positive among case herds compared with controls (OR: 1.47; 95% CI: 1.01 – 2.15). If a more restrictive cut-off value was applied to consider a herd to be positive for JD (>1 reactor), the number of JD positive herds decreased to 162 (95 cases and 67 controls), and the association was still observed ($P < 0.01$).

Also, bTB infected herds had a significantly ($P<0.01$, Wilcoxon rank test) higher number of JD-positive animals compared to control herds.

Individual-level analysis

Animals positive in both the bTB and JD diagnostic tests were found in 93 (17.5%) case herds for a total of 14,187 animals. Among those herds, the median within-herd JD prevalence was 1.59% (IQR: 0.3-2.9%). The total number of reactors to the SIT-

test, the IGRA, and the JD-ELISA was 28, 835 and 314 animals, respectively (Table 2).

Herd size ranged between 25 and 630 animals, with a median of 223 animals (IQR: 144 – 350). The predominant production type of the animals was beef (83%), followed by dairy (13%) and bullfighting (4%), and their median age was four years (IQR: 1.2 – 8.2). The median number of SIT tests performed per animal in the previous year was two (range: 1-5), with the most recent day in which the SIT test was performed ranging between 80 and 398 days (median = 183 days) before the sampling day.

The herd random effect used for the multivariable analysis included no covariates given that their addition did not improve the DIC of the null model (2828) (Table 3). Time-since-last-SIT, number-of-SIT-tests, and age-category were the animal-level variables selected in the univariable models (Table 2) and were also maintained in the multivariable model.

Animals tested more than three times in the previous year, in which the last intradermal assay was performed <3 months before the JD's testing and older than 12 months had a higher probability of testing positive in the JD-ELISA (Table 4).

The model converged well, as indicated by the visual inspection of the chains and the Gelman-Rubin^R estimates <1.01 for all parameters. Collinearity between variables was not detected, and no two-way interactions were included in the final model.

2.5 Discussion

The effect of the inoculation of PPDb during bTB-intradermal testing on the results of subsequent bTB diagnostic assays (cellular and humoral immune response tests) in both bTB-infected and bTB-free animals has been well documented and is generally accepted (Casal et al., 2017, 2014; Green et al., 2009; M V Palmer et al., 2006; W Ray Waters et al., 2015). However, reports on the effect of PPDb inoculation on the diagnostic response for JD are conflicting (Alvarez et al., 2009; Alicia Aranaz et al., 2006; Brito et al., 2014; Dunn et al., 2005; W. Lilenbaum et al., 2009; Walter Lilenbaum et al., 2007; Mosavari, Geravand, Tadayon, & Keshavarz, 2016; Varges, Marassi, Oelemann, & Lilenbaum, 2009). As with bTB, JD has its own physiopathological characteristics that hamper its diagnoses, such as its long incubation period and the delayed antibody response (Rangel et al., 2015; Sweeney, 2011). In addition, JD is a non-notifiable disease and therefore control of the disease is voluntary, resulting in limited knowledge of its distribution (Søren Saxmose Nielsen & Toft, 2009; Pearce et al., 2008).

In our study, we aimed to elucidate the association between bTB and JD infection by assessing the responses to diagnostic tests in animals from naturally coinfected herds and bTB free herds in an endemic bTB region of Spain. To address the effect of the main herd-level risk factors associated with bTB (Humblet et al., 2009), each case was matched with a control (1:1) based on (a) production type, (b) herd size, and (c) geographic proximity. Our results at the herd level analysis indicated a marginal association between the classification based on both the bTB and JD diagnostic tests. However, this association was stronger when a more restrictive (specific) cut-off was

used to classify a herd as JD positive. Additionally, when the number of reactors in the JD-ELISA from bTB infected and free herds were compared, a strongly significant difference was observed between both categories of infection. There are three non-exclusive reasons that might help to explain, at least in part, those results. First, JD herd-prevalence may be truly higher in bTB-infected herds compared to bTB-negative herds due to certain shared risk factors, such as poor management and poor biosecurity, that would lead to a higher risk for both diseases (Humblet et al., 2009; Rangel et al., 2015; Singh, Chauhan, & singh, 2016). Second, the sensitivity of the JD-ELISA may be higher in animals subjected to the more frequent skin testing in bTB infected herds when compared to OTF herds tested only once annually, (A.E. Kennedy et al., 2017; Thom et al., 2004) as discussed in the analysis at the individual level (see below). Third, a decrease in the JD-ELISA specificity at the herd level as well as a higher number of JD reactors in bTB infected herds. This lack of specificity might be due to the more frequent PPDb inoculation carried out in bTB infected herds, or due to the increase in sensitivity in the JD-ELISA while applying the same cut-off in herds with a positive and negative bTB-status (Aideen E. Kennedy et al., 2014; W. Lilenbaum et al., 2009).

The results obtained in the individual level-analysis supported the second and third hypotheses outlined above, since cattle that had been tested within the previous 90 days (three months) and that received more than three bovine PPD inoculations in the previous year had 5.00 (95% CI 1.43-10) and 1.43 (95% CI 0.26-10) higher odds of testing positive in the JD-ELISA, respectively (Table 4). The shortest possible interval between two consecutive SIT tests in a herd in Spain is 60 days (64/432/EEC,

1964) and consequently, the number of animals in which the previous SIT test had been conducted within the previous 90 days was small (510/14187, 3.6%). Notwithstanding, a significantly higher proportion of JD positive animals was observed in this cohort of animals (15.0% vs. 1.95% in the remaining 13677 animals, Table 2). When the animals that received the previous SIT test between 90 and 365 days were included in the analysis, the observed differences were no longer significant. These results suggest that the possible anamnestic response induced by the inoculation of PPDb would be of short duration vanishing at posterior times, as was previously described (de la Rua-Domenech et al., 2006; Monaghan et al., 1994). An enhanced *M. bovis*-specific antibody response after PPDb inoculation has been reported for bTB-serological tests during a similar time frame (7 to 60 days post-inoculation) (Casal et al., 2014; M V Palmer et al., 2006; Irene Schiller et al., 2010). Given the crude extract of MAP used as a coating in the JD-ELISA , this increased immune response could have also an effect in the JD-immune response resulting in the improvement of antibody detection by JD-ELISA under field conditions.

Alternatively, the increase in the rate of JD reactors in bTB infected herds after the inoculation of PPDb could be due to JD-bTB cross-reactivity, so that animals positive in the JD-ELISA would be infected with *M. bovis* instead (W. Lilenbaum et al., 2009; Walter Lilenbaum et al., 2007; Seva et al., 2014). Additionally, experimental studies showed an increase of JD-specific antibodies during the first 50 and 100 days post- *M. bovis* inoculation, although could be an effect of the presence of maternal antibodies (Eda et al., 2006; Speer et al., 2006).

Yet, when the individual results from the SIT or IGRA were analyzed, a lack of association with JD positivity was observed, in contrast with previous studies (Alvarez et al., 2008; Brito et al., 2014; Aideen E. Kennedy et al., 2014; Walter Lilenbaum et al., 2007; Seva et al., 2014). This could indicate that JD reactors were therefore not infected with *M. bovis*, and therefore positivity in the JD-ELISA would not be due to cross-reactivity in *M. bovis* infected animals. However, this result should be interpreted with care since our ability to detect an association between the responses in the SIT test and the JD-ELISA is hampered by the low number of SIT reactors observed in the case herds (Table 2). This is typical of infected herds subjected to repeated skin testing in which reactors have been removed in previous herd-tests, and there is an increased likelihood of finding infected animals in the pre-allergic state (Alvarez et al., 2012). Alternatively, the lack of association could be due to animals being in an anergic stage in which cellular tests no longer have the ability to detect infected animals and in which only the (humoral) antibody response remains (de la Rua-Domenech et al., 2006; S.S. Nielsen & Toft, 2008).

The high risk of JD-seropositivity in animals inoculated four or more times within the previous 12 months as observed in this study could be the result of increased cytokines levels after repeated SIT inoculations stimulating T-lymphocytes with PPDb, and indirectly increasing JD-ELISA sensitivity (Coad et al., 2010; Doherty et al., 1996; Radunz & Lepper, 1985; Thom et al., 2004; W Ray Waters et al., 2015).

The final model indicated that age was a risk factor for JD-ELISA positivity, which was expected and has been reported in the literature, given that the humoral response against MAP is typically observed at later and more advanced stages of the disease

and therefore in older animals (Kostoulas, Browne, Nielsen, & Leontides, 2013; Machado et al., 2018; Søren Saxmose Nielsen, Toft, & Okura, 2013).

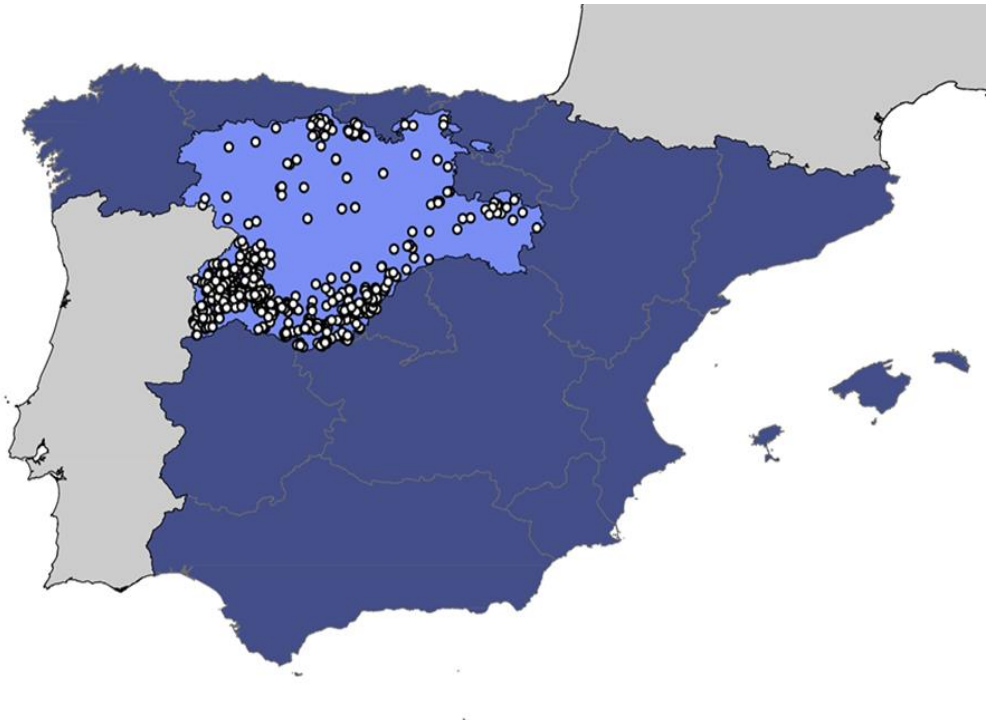
The low number of reactors to all tests detected in both case and control herds limited the power of our study (Table 2). In addition, the true JD-status of the herds was determined using the JD-ELISA, that has limited sensitivity (range 0.21-0.94, mean 0.46) and specificity (range 0.4-1.0, mean of 0.96) (Ayele, Machackova, & Pavlik, 2001; Eda et al., 2006; Mckenna et al., 2005, 2005). However, the assay has been applied successfully for the screening of JD in cattle under field conditions (Gilardoni, Paolicchi, & Mundo, 2012; S.S. Nielsen & Toft, 2008). In addition, when herd accuracy was calculated (©2018 Ausvet) using a more specific cut-off value (>=2 JD-ELISA positive animals) and discarding those potential JD false positive herds, herd sensitivity was lower, still, results showed a stronger association between bTB-infected and JD-positive herds ($P<0.012$) than the previous analysis, which supported our conclusions regardless of the uncertainty of true herd status.

In conclusion, we demonstrated here that there is an association between the herd-level bTB status and the results obtained in a JD-ELISA and that at the individual level an increased chance of positivity in the JD-ELISA occurs when animals have been subjected to frequent (>3 within a year) and recent (within 90 days) PPD inoculations as part of the SIT test. These results, together with the lack of association between the individual JD and bTB diagnostic results, suggest that repeated bTB skin testing could have a booster effect that increases the sensitivity of JD serological tests. Further studies would be required in order to confirm the true JD status of seropositive animals. Our results demonstrate the interaction between bTB repeated

testing and JD individual and herd-level results and are applicable for the evaluation of surveillance and control program for two of the most important endemic diseases affecting cattle in Spain.

2.6 Figures

Figure 6: Location of the herds studied in Castilla y Leon (CyL) region in Spain (white dot).



2.7 Tables

Table 1: The layout of case-control herds sampled to estimate the association between bTB status and JD-ELISA results.

		bTB free (controls)		
		JD positive	JD negative	Total
bTB infected (cases)	JD positive	79	68	147
	JD negative	46	71	117
	Total	125	139	264

Table 2: Association between the individual level variables and the result in a JD ELISA determined using a univariable regression model with the herd as a random effect. Median odds ratio and 95% Posterior Probability Interval (95%PPI) for each category, and the number and percentage in each category of JD-positive results.

Epidemiological Factor	**Categories (N)**	**JD positives (%)**	**Median**	**95% PPI**
Time since last SIT	<3 months (510)	47 (14.9)	(REF)	
	3-6 months (6155)	123 (39.2)	-1.4	-2.1 – (-0.7)
	>6-9 months (6448)	116 (36.9)	-1.5	-2.2 – (-0.8)
	>9 months (1074)	28 (8.9)	-1.0	-1.9 – (-0.1)
SIT result	No (14,159)	312 (99.36)	(REF)	
	Yes (28)	2 (0.64)	1.0	-0.9 – 2.5
IFN-γ result	No (13,352)	293 (93.3)	(REF)	
	Yes (835)	21 (6.7)	0.0	-0.5 – 0.4
Number of SIT tests (previous year)	>3 (1420)	11 (3.5)	(REF)	
	1 (415)	178 (56.7)	-0.3	-1.5 – 0.8
	2 (8732)	59 (18.8)	-0.7	-1.3 – 0.0
	3 (3620)	66 (21.0)	-0.8	-1.5 – 0.0
Age category	<1 year (2906)	78 (24.8)	(REF)	
	1-4 years (4331)	95 (30.2)	1.6	1.1 – 2.2
	>4-9 years(3972)	56 (17.8)	2.3	1.7 – 2.9
	>9 years (2978)	85 (27.2)	2.1	1.5 – 2.8

Table 3: Categorization of the covariates assessed at the herd level with the posterior fixed effects regression coefficients Median (odds ratio), standard deviation (sd), 95% Posterior Probability Interval (95% PPI), and Deviance Information Criterion (DIC) for the univariable analysis for the random effect model.

Epidemiological Factor	**Categories (%)**	**Median**	**sd**	**95% PPI**	**DIC**
Herd size	>200 anim (51)	(REF)			
	101-200 anim (35)	0.0	0.2	-0.5 – 0.5	
	51-100 anim (11)	-0.3	0.3	-1.0 – 0.3	
	<51 anim (3)	0.3	0.4	-0.6 – 1.1	2887
Production type	Beef (83)	(REF)			
	Dairy (13)	- 0.1	0.4	-0.8 - 0.6	
	Bullfighting (4)	- 0.5	0.6	-1.7 -0.7	2887

Table 4: Final individual animal model posterior fixed effects Odds Ratio Median, standard deviation (sd), and 95% posterior probability interval (95% PPI).

Parameter	**Category(N)**	**Median**	**sd**	**95% PPI**
Animal age	<1 year (2906)	(REF)		
	1-4 years (4331)	4.7	1.7	2.6 – 9.1
	>4-9 years(3972)	9.6	3.4	5.5 – 18.4
	>9 years (2978)	8.4	3.1	4.7 – 16.5
Time since last SIT	<3 months (510)	(REF)		
	3-6 months (6155)	0.2	0.2	0.1 – 0.7
	>6-9 months (6448)	0.2	0.2	0.1 – 0.8
	>9 months (1074)	0.3	0.5	0.1 – 1.8
Number of SIT tests (previous year)	>3 (1420)	(REF)		
	1 (415)	0.7	1.1	0.1 – 3.8
	2 (8732)	0.8	0.4	0.3 – 1.8
	3 (3620)	0.7	0.3	0.3 – 1.6

CHAPTER 3 – Diagnostic interaction between bovine tuberculosis (bTB) and Johne's disease in bTB highly prevalent dairy farms of Uruguay

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3.1 Chapter summary

The consolidation of the dairy industry, with an increase in sizes, density, and productivity of the herds, was associated with unprecedented bovine tuberculosis (bTB) prevalence levels in dairy herds in Uruguay, where Johne's disease (JD), another mycobacterial disease, is also prevalent. Here, we aimed to characterize the association between bTB- and JD-diagnostic results in two heavily bTB- and JD-co-infected dairy herds. Results from bTB-intradermal tests and JD-ELISA in 686 cows indicated a significantly ($P<0.001$) higher frequency of bTB-positive animals in the JD-positive population, in which a significantly lower agreement between the caudal and cervical comparative intradermal tests was observed, compared to the JD-negative population. These findings suggest a significant association between the detection of these mycobacterial diseases, that may affect the performance of the routine bTB diagnostic tests performed in dairy herds in Uruguay.

Keywords: Caudal Fold Tuberculin; Comparative Cervical Tuberculin, ELISA; *Mycobacterium avium* subsp. *paratuberculosis* (MAP), Dairy cattle.

3.2 Introduction

Bovine tuberculosis (bTB) is a worldwide-distributed chronic infectious disease of cattle caused mainly by infection with *Mycobacterium bovis (M. bovis)*, which represents a threat to animal and public health. The annual number of bTB-positive detected herds, the within-herd prevalence in infected herds, and the time from detection-to-control[1] has increased over the last decade in Uruguay, despite measures implemented as part of the national bTB-control program. Briefly, dairy animals are annually tested with the caudal fold tuberculin test (CFT), and reactors are confirmed with the comparative cervical tuberculin test (CCT). CCT reactors are sent to slaughter, and the complete herd is re-tested every 60 to 120 days until the herd has two consecutive negative herd-tests are achieved. Challenges in controlling the disease may be explained, at least in part, by the consolidation of the dairy industry[2], given that large dairy herds (>360 animals) with frequent movement of animals (>44 individuals annually) have become relatively common in the country. Those unusually large dairy farms have been found to experience a high risk of bTB-breakdown, what could be related to the limited efficacy of control strategies in place, that were initially designed for a more traditional dairy production standards characterized by relatively small farms, with less animal density, less individual production pressure, and infrequent movements (Picasso et al., 2017).

Insufficient accuracy of the bTB-diagnostic tests routinely used in Uruguay (caudal fold test –CFT- followed by the comparative cervical test –CCT- for

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[a] See: http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation
[b] See https://descargas.mgap.gub.uy/DIEA/Anuarios/Anuario2018/Anuario_2018.pdf

confirmation of reactors), represents an additional constraint to the success of the bTB control program. This lack in performance may be explained in part by the cross-reactivity with other mycobacterial infections, such as *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the etiological agent for Johne's disease (JD), particularly in the case of the CFT(Brito et al., 2014). In Uruguay, JD is widespread in dairy cattle, with a reported within-herd prevalence of 2.5% in 2015 (Suanes et al., 2018).

In the current changing scenario of the Uruguayan dairy industry, with untraditional large herds, frequent animal movements, and widespread occurrence of JD, there is a need to assess the performance of the bTB-diagnostic strategies (CFT, and CCT), and their potential interaction with JD-diagnosis, with the ultimate objective of evaluating and informing the design of the control plan for the disease. Here, we aimed to characterize and estimate the association between bTB- and JD-diagnostic results in two farms with the recently identified bTB risk factors in which both diseases were present at high prevalence.

3.3 Methods

We randomly sampled 686 Holstein cows (>24 months) from two large (~1000 dairy females, >75th percentile for the country[2]) dairy herds from one of the largest dairy companies in Uruguay. These two herds had been bTB-infected since 2013, had a history of JD-seropositive results and animals with chronic diarrhea, were located in the same geographic region, mingled their animals frequently and

systematically, had similar management practices, and were subjected to similar bTB-control measures, which are mandatory for bTB-infected herds in Uruguay[3].

Selected animals were subject to bTB-intradermal testing which involves the inoculation of purified protein derivate from *M. bovis* (PPDb) for CFT, and from *M. bovis* and *M. avium* (PPDa) for the CCT. The CFT was performed in all animals, and those with a palpable increase in skin thickness or with local clinical signs of inflammation 72hrs post inoculation were re-tested using the CCT within the following 10 days. Animals with >4 mm increase the difference in skin thickness between the PPDb- and PPDa- inoculation site after 72hs were considered bTB-infected[2] . Additionally, serum samples were collected from the coccygeal vein of all animals when the CFT was interpreted to perform a JD-indirect Enzyme-Linked Immunosorbent Assay (ELISA) (ID.Vet, Montpellier, France) following the manufacturer recommendations; animals with a sample-to-positive ratio result (S/P) ≤ 0.6 were considered negatives and otherwise were classified as positives.

3.4 Results and discussion

Most animals (427/686, 62.24%) were positive to the CFT, and of that, 58.08 % (248/427) were also positive to the CCT. In addition, 44.16% (303/686) animals tested positive to the JD-ELISA, confirming the high bTB- and JD- prevalence. Interestingly, the proportion of bTB-positive animals (to both CFT and CCT) was significantly (P<0.001) higher among JD-reactors compared to JD-negative cattle (Table 1). The degree of diagnostic interference in bTB- and JD-diagnostic tests in

[c] See: http://www2.mgap.gub.uy/portal/page.aspx?2,dgsg,dgsg-legislacion-sanitaria

animals infected with either or both pathogens under field conditions is still not fully understood(Brito et al., 2014; Alicia Aranaz et al., 2006; Dunn et al., 2005). Results here may indicate that animals infected with one of the diseases would develop a cross-reactive immune response to the other disease, increasing the sensitivity of the other test or reducing its specificity(Roupie et al., 2018; Alvarez et al., 2009; Walter Lilenbaum et al., 2007), what may have happened here, with a decreased specificity of the JD-ELISA(Dunn et al., 2005). Another potential explanation may be an increased individual susceptibility (or resistance) to mycobacterial infections in which animals that are infected with one disease are/become more susceptible to the other.

The agreement between the two bTB intradermal tests (CFT and CCT) was fair in the JD-ELISA-positive animals (kappa-coefficient= 0.34, CI:0.25-0.42) and moderate in the JD-ELISA-negative population (kappa-coefficient = 0.52, CI:0.44-0.59), with a lower agreement in the JD-positive animals than the overall agreement observed (Table 1). The main source of disagreement between the results in the two intradermal tests in the JD-positive group was the higher proportion of CFT-positive/CCT-negative results (0.31 compared with 0.22 among the JD-negative animals). Such difference suggests that JD-seropositivity has an impact on the result of the skin tests, by either reducing CFT-specificity or CCT-sensitivity. Potential explanations for a higher rate of CFT-false positive results include the cross-reactivity between bTB and JD, with the subsequent CFT-specificity reduction that may only be partially resolved with the use of the CCT. A reduction in the CCT-sensitivity, on the other hand, could be explained by the induction of a greater response to the PPDa inoculation that would mask the PPDb reaction, leading to CCT-false negative results

in bTB-infected animals (Hope et al., 2005). The potential impact of this phenomenon in smaller infected herds in which the number of reactors would be lower should be assessed. Still, in a scenario of a high prevalence of bTB as the one evaluated here the reduction in the CCT-sensitivity (and therefore of its negative predictive value) is the most concerning possibility, and highlights the necessity to re-evaluate the use of testing in series to control bTB in high bTB-prevalent and JD-coinfected herds in Uruguay.

This study is the first to characterize the diagnostic interaction between bTB and JD, the two most important mycobacterial diseases in Uruguayan cattle, in high prevalence, coinfected herds. Most importantly, our findings suggest that evaluating the distribution of both diseases in high prevalence bTB-infected herds in Uruguay may be important to facilitate disease control and eventual eradication.

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3.5 Tables

Table 5: Bovine tuberculosis and Johne's disease (JD) diagnostic results in dairy cows selected.

JD-ELISA	Number of samples (%)	Number of cows positive to CFT[a] (%)	Number of cows positive to CCT[b] (%)	Cohen's kappa[c] (95%CI)
Positive	303(44.16)	260 (85.81)	167(55.11)	0.34 (0.25 – 0.42)
Negative	383(55.84)	167 (43.60)	81(21.81)	0.52 (0.44 – 0.59)
Total	686	427 (62.24)	248(36.15)	0.46 (0.51 – 0.56)

[a] Caudal Fold Tuberculin test (CFT)

[b] Comparative Cervical Tuberculin test (CCT)

[c] Agreement between CFT and CCT.

CHAPTER 4 – Modeling the accuracy of two in-vitro bovine tuberculosis tests using a Bayesian approach

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4.1 Chapter summary

The accuracy of new or alternative diagnostic tests is typically estimated in relation to a well-standardized reference test referred to as a gold standard. However, for bovine tuberculosis (bTB), a chronic disease of cattle that affects animal and public health, no reliable gold standard is available. In this context, latent-class models implemented using a Bayesian approach can help to assess the accuracy of diagnostic tests incorporating previous knowledge on test performance and disease prevalence. In Uruguay, bTB-prevalence has increased in the past decades partially because of the limited accuracy of the diagnostic strategy in place, based on intradermal testing (caudal fold test, CFT, for screening and comparative cervical test, CCT, for confirmation) and slaughter of reactors. Here, we evaluated the performance of two alternative bTB-diagnostic tools, the interferon gamma assay, IGRA, and the enzyme-linked immunosorbent assay (ELISA), which had never been used in Uruguay in the absence of a gold standard. In order to do such, animals from two heavily infected dairy herds and tested with CFT-CCT were also analyzed with the IGRA using two antigens (study 1) and the ELISA (study 2). The accuracy of the IGRA and ELISA was assessed fitting two latent-class models: a two test-one population model (LCA-a) based on the analysis of CFT/CFT-CCT test results and one in-vitro test (IGRA or ELISA), and a one test-one population model (LCA-b) using the IGRA or ELISA information in which the prevalence was modeled using information from the skin tests. Posterior estimates for model LCA-a suggested that IGRA was as sensitive (75-78%) as the CFT and more sensitive than the serial use of CFT-CCT. Its specificity (Sp: 90-96%) was superior to the one for the CFT and equivalent to the use of CFT-CCT. Estimates from LCA-b models consistently yielded lower posterior Se estimates

for the IGRA but similar results for its Sp. Estimates for the Se (52% 95PPI: 44.41, 71.28) and the Sp (92% PPI: 78.63, 98.76) of the ELISA were however similar regardless of the model used. These results suggest that the incorporation of IGRA for detection of bTB in highly infected herds could be a useful tool to improve the sensitivity of the bTB-control in Uruguay.

4.2 Introduction

The accuracy of diagnostic tests has been traditionally estimated by comparing the test results with those of a reference test, sometimes referred to as the gold standard, which unequivocally indicates the true status of an individual (infected/not infected). In the absence of such a reference test, latent class analyses based on Bayesian methods provide an alternative strategy for evaluation of diagnostic tests when the true status of the individual is unknown. The use of this approach in the context of veterinary medicine has been described elsewhere (Branscum, Gardner, & Johnson, 2005). Briefly, the use of latent class analyses based on Bayesian methods involves the combination of previous knowledge on test performance (when available) with the evidence provided by newly collected data to obtain a posterior estimate on test performance and disease prevalence, often achieved through Monte Carlo simulations using Gibbs sampling (D. Spiegelhalter, Thomas, Best, & Gilks, 1996). The prior knowledge on test performance is typically obtained through the review of the scientific literature and/or the elicitation of expert opinion (Suess, Gardner, & Johnson, 2002). Methodologies to elicit expert opinion have been described elsewhere (Suess et al., 2002).

Use of latent class models in veterinary epidemiology has increased in the past decades, particularly for the assessment of diagnostic tests for chronic and complex diseases for which gold standard tests are not available, such as bovine tuberculosis (bTB) (Al-Mouqatea et al., 2018; Alvarez et al., 2012; de la Cruz et al., 2018; Valerie-Beau Pucken et al., 2017).

Bovine tuberculosis, mainly caused by infection with *Mycobacterium bovis* (*M. bovis*), is an important chronic disease of cattle that causes a substantial impact on animal and public health, and that imposes a significant economic burden associated with its control and international trade restrictions (Collins, 2006; Zinsstag, Schelling, Roth, & Kazwala, 2008).

Control programs worldwide are based on test and removal of positive animals or, in some cases, complete herds (OIE, 2009). In Uruguay, the bTB-national program involves serial intradermal testing (caudal fold test –CFT- followed by the comparative cervical test –CCT- for confirmation) of all dairy herds annually for the detection of infected animals and its posterior removal (Casas Olascoaga, 2013; Picasso et al., 2017). In the past decade, the number of bTB-positive dairy herds detected every year, the within-herd prevalence in infected farms, and the time from outbreak detection to control has increased in Uruguay despite measures implemented as part of the national bTB control program (Picasso et al., 2017; WAHIS_OIE, 2014). The evolution of the dairy industry in the country, characterized by an increase in herd sizes and production intensification, has been associated with the limited success of bTB-control in recent years (Picasso et al., 2017; Picasso-Risso et al., 2019). Additionally, insufficient sensitivity of bTB diagnostic tests may also

contribute to the persistence of potentially infectious individuals in the herd that can further spread the disease within and between herds (Alvarez, Bezos, et al., 2014).

In Europe the use of the interferon-gamma release assay (IGRA) in parallel with the skin test has been incorporated in many eradication programs to maximize diagnostic sensitivity (Council Directive 64/432/EEC, 1964) (EFSA (European Food Safety Authority), 2012; EFSA Panel on Animal Health and Welfare (AHAW) et al., 2017). Other tests based in the detection of specific antibodies (such as the enzyme-linked immunosorbent assay, ELISA) have been developed and proven useful for detection of specific subpopulations of *M. bovis*-infected animals that may not react to the skin test, although their field use has been mostly limited so far to experimental purposes (Casal et al., 2014; W R Waters et al., 2011; W. R. Waters et al., 2006; Konstantin P. Lyashchenko, Pollock, Colangeli, & Gennaro, 1998; K. Lyashchenko et al., 1998; Radunz & Lepper, 1985). Characterization of the performance of alternative diagnostic tools (IGRA and ELISA) in Uruguay may help to design strategies for the improvement of the diagnostic sensitivity in high bTB-prevalence infected dairy herds, currently a priority for the control and eradication of bTB in the country.

Here, we aimed to estimate the accuracy of two commercial assays for in-vitro diagnosis of bTB that had never been used in Uruguay, namely an IGRA (using two alternative antigens) and an antibody-based ELISA, fitting two different latent-class models in a Bayesian framework. Results from this study will help to quantify the potential impact that alternative diagnostic strategies may have in improving the effectiveness of the bTB-control program in Uruguay.

4.3 Methods

We followed the STARD-BLCM guidelines to describe the materials and methods in our study (Kostoulas et al., 2017).

Study design

Two studies (referred to as study 1 and study 2) using a "single-gate" diagnostic design were performed to evaluate the performance of the in-vitro bTB-diagnostic assays. In brief, a "single-gate" study includes positive- and negative-infected animals selected from a single population, in contrast to a "two-gate" study in which cases and controls are selected using a diverse inclusion criteria, which leads to two different populations (Rutjes, Reitsma, Vandenbroucke, Glas, & Bossuyt, 2005)(Rutjes et al., 2005).

Source populations

Sampling for both studies was carried in 2016, and included 121 and 279 Holstein cows for studies 1 and 2, respectively. All animals were selected from two commercial dairy herds belonging to the same company (with similar management practices and that frequently and systematically mingle their animals) located in the Department of Florida. Both herds were bTB positive since 2013. The two herds were subjected to the intradermal test as regulated by the national bTB-control program in Uruguay for dairies based on the status of the herd (MGAP, 1989). In addition, blood (study 1) or serum (study 2) samples were drawn from the selected animals (Fig 1).

Sampling and diagnostic assays

All dairy > 12-month animals were tested using the CFT as a screening test, involving the intradermal inoculation of a purified protein derivate from M. bovis (PPDb) in the caudal area. Animals with an increase in skin thickness and/or presence of in-situ clinical signs of inflammation 72hs post inoculation were considered reactors and subjected to the CCT for confirmation within the following seven days. In this test, two PPD inoculations from M. bovis (PPDb) and M. avium (PPDa) are performed in the cervical area. When the difference in skinfold thickness in the PPDb inoculation site was ≥4 mm than the PPDa animals were considered infected and culled.

Blood or serum samples were collected from the coccygeal vein of cows enrolled in studies 1 and 2, respectively, after the results of the serial CCT test were assessed (if applicable). Samples were maintained at environmental temperatures (20 to 25 ºC) until arrival to the official veterinary diagnostic laboratory (Miguel C. Rubino) within the first 8 hours post extraction to perform the IGRA (blood) or the ELISA (serum).

In study 1, blood samples were stimulated with specific antigens as described elsewhere (P. R. Wood et al., 1990). All samples were divided into five aliquots and incubated for 18h with pokeweed mitogen, PBS (blank), PPDa, PPDb and an antigenic cocktail formed by the early secretory antigenic target-6 (ESAT-6) and the culture filtrate protein 10 (CFP-10), two highly specific M. bovis antigenic proteins (Vordermeier et al., 2001)(Vordermeier et al., 2001). Samples were then centrifuged, and the supernatant was analyzed using the Bovigam 2.G (Prionics, Schlieren-Zurich,

Switzerland) according to the manufacturer's recommendations. Two criteria based on different sets of antigens were applied to classify animals as positive; for criteria A (IGRAb) animals were considered positive if the optical density (OD) obtained after stimulation with PPDb (ODPPDb) minus the OD of the aliquot stimulated with PBS (ODPBS) was ≥0.1 and ODPPDb - ODPBS ≥0.1; in the case of criteria B (IGRAc), animals were classified as positive when ODcocktail - ODPPDb was ≥0.1. For study 2, a commercial ELISA (IDEXX Laboratories, Westbrook, ME) was used to detect MPB83 and MPB70 bTB specific antibodies as described elsewhere (W R Waters et al., 2011). Animals with an S/P ratio ≥ 0.3 were considered positive and negative if else as recommended by the manufacturer.

Statistical models

Latent-class models were used to estimate diagnostic test accuracy (sensitivity –Se-, and specificity -Sp-) of the IGRA using the different antigens (IGRAb and IGRAc) and the ELISA in the absence of a gold standard assay (Branscum et al., 2005; Ian A Gardner, Stryhn, Lind, & Collins, 2000). Samples collected were assumed to originate from a single population given they were drawn from herds belonging to the same company with similar animal health status regarding bTB and similar production management standards.

For each study (1 and 2), two different models were used alternatively: a two dependent tests-one population model (LCA-a) using the results from the skin test (CFT or CFT-CCT) and one of the in-vitro tests (IGRA or ELISA), and a one test-one population model (LCA-b) analyzing the results of the in-vitro tests separately (Fig. 1).

Conditional correlation coefficients for the Se (rhoD) and Sp (rhoDc) were included in the LCA-a models as described elsewhere (Ian A Gardner et al., 2000). We assumed results from the tests were conditionally dependent because results from diagnostic tests targeting a similar biological phenomenon, such as the intradermal tests and the IGRA (Pollock et al., 2005), are likely dependent (Ian A Gardner et al., 2000; Georgiadis, Johnson, Gardner, & Singh, 2003). Similarly, and although the ELISA is based on the detection of the humoral immune response in the infected animals, there is a relationship between the initial predominant cellular-mediated immunity and the posterior humoral immunity observed as disease progresses in the animal (de la Rua-Domenech et al., 2006), so results from the skin test and the ELISA were also assumed to be conditionally dependent.

Beta prior distributions for the Se and Sp of the CFT, CFT-CCT, IGRAb, IGRAc, and ELISA were chosen according to previous reports (Table 1 & Supplementary table 1). Distributions were fitted using Beta buster version 1.0 (http://252s-weblive.vet.unimelb.edu.au:3838/users/epi/beta.buster/). More informative distributions were used for the Se and Sp of the CFT-CCT due to the availability of Uruguay-specific information compared with those used for the in-vitro assays since most references for those originating from other countries with a different experience in the use of these techniques (Table1).

For the LCA-a (two-dependent-test) models, prevalence priors were formulated from expert opinion following procedures described elsewhere (Suess et al., 2002). For the LCA-b (one-test) models, prior distributions for prevalence were formulated using the results from the CFT-CCT as described previously (Rogan &

Gladen, 1978). Briefly, we simulated the true prevalence distribution using the Rogan-Gladen estimation method to correct for the imperfect Se and Sp of the CFT-CCT (assumed to follow beta distributions as mentioned before) (Table 1) through 5,000 iterations in an Excel spreadsheet (Microsoft Office Professional Edition, 2016) using @Risk software version 7.0.0 (Palisade Corporation 2015). The outputs from the simulations were used to fit a beta distribution that was used as the prevalence prior to LCA-b models.

Three Markov chain Monte Carlo runs were implemented per model to visually assess convergence (also tested using the Gelman-Rubinˆ R statistic) (Andrew Gelman & Rubin, 1992). Models were run for 7,500 iterations for computing posterior estimates after an initial burn-in of 2,500 samples. To eliminate potential autocorrelation, we applied thinning and selected one every 10 consecutive samples. Latent-class models were fitted using OpenBUGS 3.2.2 (Lunn et al., 2009) via the R2OpenBUGS package (Sturtz, Ligges, and Gelman 2005) from the R 3.2.4 software. The influence of the selected priors on the posteriors distributions was evaluated by comparing the initial models with a model fitted using non-informative uniform (0,1) distributions for each parameter under evaluation. The possible independence between the results of the two tests being assessed was also evaluated by fitting models that did not include correlation terms. Model fit was assessed using the deviance information criterion (DIC), and the model selection (LCA-a or LCA-b) was based on lower DIC (D. J. Spiegelhalter et al., 2002) and narrower posterior credibility intervals.

4.4 Results

Cross-tabulated dichotomous results for the combination of the intradermal tests (CFT or CFT-CCT), and the in-vitro assays (IGRAb, IGRAc, or ELISA) are presented in table 2.

The estimated posterior estimates for the Se and Sp of the diagnostic tests and the prevalence in Study 1 and 2 are shown in table 3.

Study 1. Median posterior estimates for the prevalence, Se and Sp of the intradermal tests (CFT, and CFT-CCT) using the LCA-a model were similar regardless the antigen used in the IGRA (IGRAb or IGRAc) (table 3). The median posterior IGRAb Sp estimates were slightly lower than those obtained for the IGRAc, whit higher median Sp values for the models integrating CFT-CCT as the second test as well but with the overlapping of the PPIs (Table 3).

LCA-b models consistently yielded lower Se values for both IGRAs and higher prevalence estimates compared with LCA-a models, but with similar Sp posterior estimates.

Study 2. The LCA-a model yielded higher posterior estimates for the prevalence and Se of the intradermal tests, and a markedly lower Sp posterior values for CFT compared to those observed in study 1 using the same model. ELISA Se and Sp estimate obtained using the two models (LCA-a and b) were consistent.

Conditional correlation between intradermal and in-vitro test results in infected (rhoD) and non-infected animals (rhoDc) was low, with 95%PPI including 0 in all LCA-models for study 1(table 3). However, no significant improvement was

observed in the DIC when test independence was assumed for models using IGRAs and CFT (study 1: 19.4 vs. 19.4, 19.4 vs. 19) or IGRAs and CFT-CCT (study 1: 18.1 vs. 17.5, 17.7 vs. 19.7) respectively. Interestingly, the LCA-a model from study 2 showed the highest median correlation terms for infected animals (rhoD = 11.7 and 17.05), showing a poorer fit of the model when independent-tests models were assessed (ELISA and CFT DIC:19.4 vs 24.7, ELISA and CFT-CCT DIC:24.4 vs 31.9), although 95% PPI included 0.

The sensitivity analysis revealed that results obtained using LCA-a models for study 1 were not affected (changes <10.5%) by the use of weakly informative priors (Suppl table 2). However, various parameters were severely affected (changes>10.5% when weakly informative priors were used) by choice of priors in the remaining models/studies. Results were most affected when LCA-a models were applied in study 2. The use of uniform distributions for the Sp of the in-vitro assays in both studies resulted in 17.8 to 38.2% decreased posterior median Sp values. Similarly, use of uniform priors for the prevalence resulted in a >20% reduction in posterior estimates of study 1 using the LCA-b model (76.5 to 54.3 and 65.4 to 52.2), and an increase in the Se estimates for IGRAb and IGRAc.

All models reached convergence as indicated by the visual inspection of the Markov chains and the Gelman-Rubinˆ R statistic (<1.002) for all parameters.

4.5 Discussion

Due to the increasing number of bTB- infected herds in Uruguay (Animal Health Bureau, Uruguay -DSA MGAP-), the need for early and accurate detection,

isolation and removal of infected animals from a herd is crucial when the whole herd-culling is not an economically or socially sustainable option. Here, we aimed to assess the performance of bTB-in-vitro assays under field conditions for the first time in Uruguay with the ultimate goal of improving current bTB diagnostic strategies for chronic and high prevalence infected dairy herds.

In order to estimate the performance of the in-vitro assays evaluated here we used LCA, a suitable analytical approach when no reliable gold standard is available (Branscum et al., 2005; Enøe, Georgiadis, & Johnson, 2000; I.A. Gardner, 2002), as it is the case for bTB (Alvarez et al., 2012; de la Cruz et al., 2018; Praud, BOSCHIROLI, MEYER, GARIN-BASTUJI, & DUFOUR, 2015). We fitted two different latent-class models using prevalence priors based on expert opinion or diagnostic test results in order to evaluate the potential impact of a given methodological approach. Based on DIC, models with three (Se, Sp, Prev) parameters were preferred above those with seven (Se1, Se2, rhoD, Sp1, Sp2, rhoDc, Prev). Correlation between test results were very low in all models/test pairs, what had been already described for the IGRA and single skin test (Alvarez et al., 2012; de la Cruz et al., 2018) such result is expected because the diagnostic tests evaluated here have high Sp (Branscum et al., 2005). However, the comparatively higher correlation between the ELISA and CFT or CFT-CCT estimates in bTB-infected animals (rohD) was surprising, given that the ELISA and the skin tests target different immune responses and therefore a larger degree of independence is often assumed (de la Rua-Domenech et al., 2006; Drewe, Tomlinson, Walker, & Delahay, 2010).

Prevalence priors elicited from expert opinion were considerably lower than those based on the intradermal test results (median of 0.35 vs. 0.85). That finding could explain, at least in part, the lower posterior estimates for prevalence obtained in LCA-a models compared with those from LCA-b models. The higher posterior prevalence estimates obtained using all models in both studies; along with the fact that the two sampled herds remained infected with high rates of reactors two years after this study was completed (data not shown) suggest that bTB-infection was higher than what was estimated using expert opinion in this population. Comparison of results from the two modeling approaches illustrates the potential negative consequences of basing prior distributions exclusively on expert opinion.

Interestingly, estimates for the Se and Sp of the CFT test were lower than those described for the US (Farnham, Norby, Goldsmith, & Wells, 2012b; Norby et al., 2004; D. L. Whipple et al., 1995), and more in line with Se values reported in field studies in Australia (P. Wood et al., 1991). Likewise, posterior estimates for the serial use of CFT-CCT remained in the lower end of previous estimates (Norby et al., 2004; Nuñez-Garcia et al., 2018; VanderWaal et al., 2017; Vordemeier et al., 2006). This relatively low accuracy of the intradermal tests in Uruguay suggests that the bTB-control program may suffer from limited Se in heavily infected herds, what could lead to the persistence of infected animals in the dairy cattle population over time, which, with the consolidation and intensification of the industry, may have contributed to the re-emergence of bTB observed in the last decade (Picasso-Risso et al., 2019).

Posterior estimates for the Se of IGRAb and IGRAc obtained using LCA-a models (table 3) are in agreement with previous reports suggesting IGRAs are at least

as sensitive as intradermal assays (Bezos, Casal, et al., 2014; de la Rua-Domenech et al., 2006). IGRAs have two major advantages over intradermal tests, namely, the potential for detecting false negative animals in the skin test (Coad et al., 2010, 2010; E. Gormley et al., 2004; Monaghan et al., 1994; Vordemeier et al., 2006), and the opportunity to maximize their sensitivity thanks to the anamnestic effect induced by the inoculation of PPDs when used in combination with intradermal tests (Casal et al., 2014; M V Palmer et al., 2006; Vordemeier et al., 2006). The population under study was sampled at post intradermal inoculation of the PPDb, while this time was variable, it could have contributed to an enhanced Se in agreement with previous studies in which IGRAs performance was assessed following intradermal tuberculin testing (Waters et al., 2015).

A slightly higher Sp was obtained for the IGRAc compared with the IGRAb, what could be due to the use of more specific antigens (peptide-cocktail with ESAT-6 and CFP-10) (Aagaard et al., 2006; Casal et al., 2012; Flores-Villalva et al., 2012; Vordermeier et al., 2001) although could be also a product of the different priors used for each test based on available knowledge. Interestingly, Sp of the IGRAc was equivalent to that of CFT-CCT, suggesting that the use of a single assay (IGRAc) could potentially replace serial testing with CFT-CCT for bTB screening in heavily infected dairy herds.

In conclusion, results found here, irrespective of the modeling approach followed, suggest that the use of IGRAs in Uruguay can dramatically improve the limited Se of the currently used diagnostic strategies based on skin tests, which would require numerous herd tests to eliminate the disease from heavily infected herds as the

ones analyzed here. The ELISA could also have some potential for detection of bTB-infected animals if used as an ancillary test to skin test in these populations.

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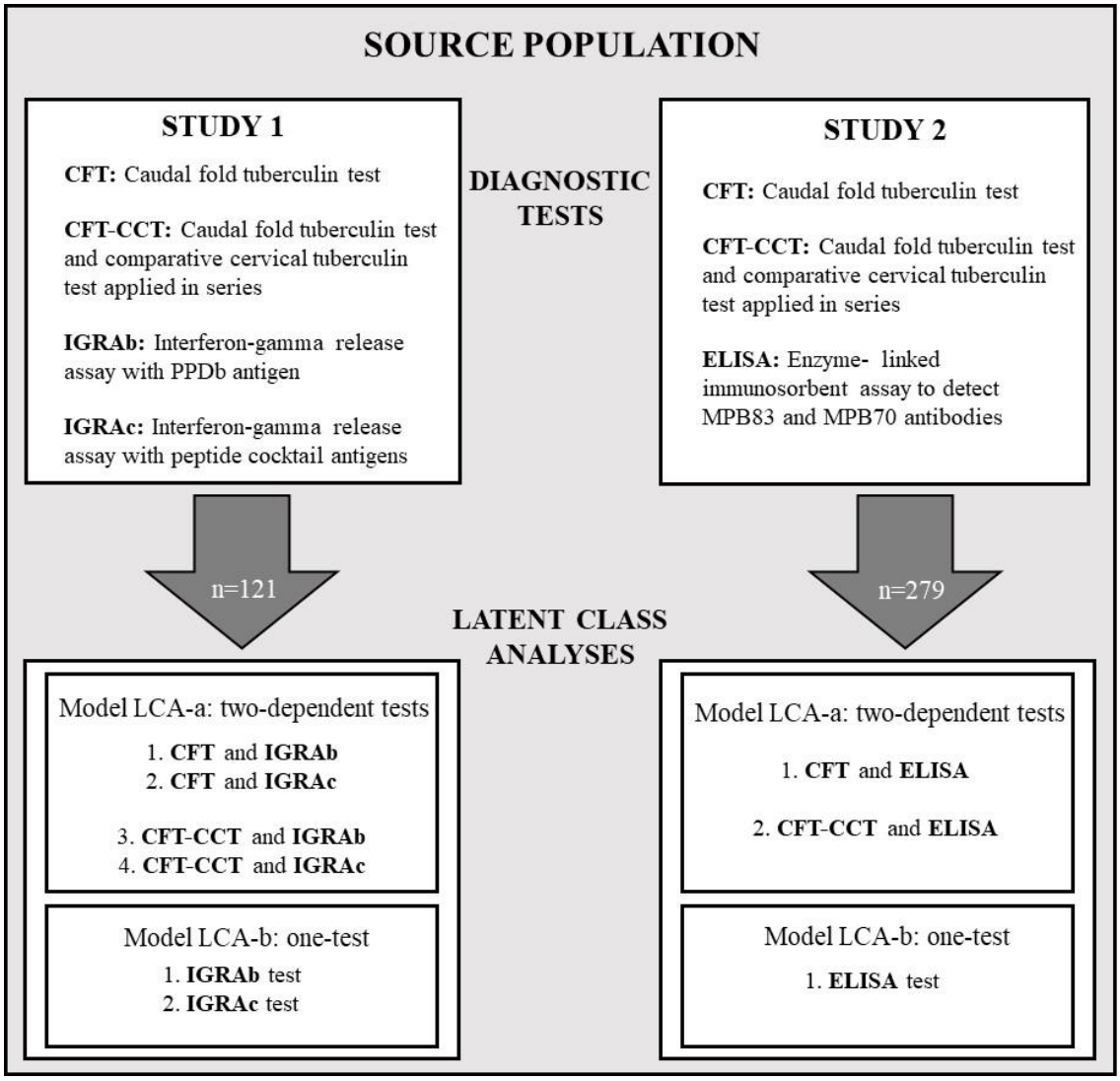
4.6 Figures

Figure 7: Schematic diagram showing the study design, with the diagnostic tests used for study 1 and study 2, and the Bayesian latent-class fitted models LCA-a and LCA-b.



4.7 Tables

Table 6: Prior estimates (Mode and 5th percentiles) for sensitivity, specificity of the intradermal tests (CFT, CFT-CCT) and in-vitro (IGRAb, IGRAc, and ELISA) bTB tests, and prevalence for the two models implemented.

Diagnostic test	**Priors estimates**			
	Sensitivity	Beta distribution	Specificity	Beta distribution
CFT	80 (>51)	α: 7.99, β: 2.75	90 (>60)	α: 8.3045, β: 1.81
CFT-CCT	53 (>46)	α: 73.81, β: 65.57	97 (>94)	α: 176.39, β: 6.42
IGRAb	83.5 (>48)	α: 5.99, β: 1.99	95 (>80)	α: 21.20, β:2.06
IGRAc	80 (>60)	α: 14.84, β: 4.46	97 (>94)	α: 176.39, β: 6.42
ELISA	57.1(>33.1)	α: 6.98, β: 5.49	95 (>81)	α: 23.25, β: 2.17
Prevalence(*)	35 (>15)	α: 3.63, β: 5.88		Experts opinion
Prevalence (+)	85 (>61)	α: 8.46, β: 1.742		CFT-estimated

(*) Prevalence priors distributions based on expert opinions used in the LCA-a

(+) Prevalence priors distributions based on results from the intradermal test (CFT) used in the LCA-b

Table 7: Cross-tabulated dichotomous diagnostic results for intradermal test (CFT, CFT-CCT) and in-vitro (IGRAb, IGRAc, ELISA) bTB- diagnostic tests.

Study	Diagnostic test		CFT+	CFT-	CFT-CCT+	CFT-CCT-	Total
1	IGRAb	Positive	35	19	26	28	54
		Negative	25	42	10	57	67
	IGRAc	Positive	34	16	24	26	50
		Negative	26	45	12	59	71
		Total	60	61	36	85	121
2	ELISA	Positive	126	3	91	38	129
		Negative	108	42	64	86	150
		Total	234	45	155	124	279

Table 8: Posterior estimates (median and 95% posterior probability interval) for CFT, CFT-CCT and in-vitro assays (IGRAb, IGRAc, ELISA) sensitivities, specificities, prevalence, and, when applicable, correlation terms (rhoD, rhoDc) distributions obtained for study 1 (121 animals) and study 2 (279 animals), applying the model 'a', or the model 'b' in chronic naturally infected dairy herds in Uruguay.

Study/ Model	**Diagnostic test**			**Posteriors estimates**				
	Test-one	Test-two	DIC	Sensitivity	Specificity	Prevalence	rhoD	rhoDc
1/a		IGRAb		75.32 (58.96, 91.63)	89.96 (77.82, 97.23)*	50.84 (33.80, 67.73)	-4.09 (-28.94, 35.07)	-2.78 (-20.70, 23.68)
	CFT		19.4	73.34 (56.88,89.44)	77.02 (58.96, 95.48)			
		IGRAc	19.4	75.73 (62.45, 88.08)	96.49 (93.85, 98.22)*	51.33 (38.11, 65.33)	-3.50 (-24.00, 24.73)	-0.33 (-7.84, 9.23)
	CFT			72.43 (58.34, 83.75)	76.23 (59.98, 93.95)			
2/a		ELISA	19.4	57.82 (48.92, 73.43)	93.76 (85.57, 98.08)	76.94 (56.97, 87.80)*	11.17 (-1.96, 29.72)	4.75 (-1.39, 27.78)
	CFT			95.48 (88.83, 98.91)	63.87 (34.15, 94.31)			
1/a		IGRAb	18.1	78.01 (62.97, 89.53)	91.43 (78.91, 98.26)	50.37 (37.38, 63.48)	-2.47 (-31.69, 29.59)	-0.48 (-8.19, 16.20)
	CFT-CCT			53.27 (45.76, 60.59)	96.19 (92.78, 98.37)			

		IGRAc	17.7	76.21 (65.35, 85.86)	96.56 (93.34, 98.52)	51.30 (40.28, 62.97)	-6.03 (-28.49, 17.95)	-0.09 (-3.42, 5.10)
	CFT-CCT			52.89 (45.66, 59.93)	96.13 (92.66, 98.32)			
2/a		ELISA	24.4	52.29 (44.96, 60.35)	92.41 (78.82, 98.48)*	79.73 (73.23, 91.80)*	17.05 (-0.26, 31.64)	-0.08 (-8.02, 16.78)
	CFT-CCT			60.44 (54.45, 66.59)	96.14 (92.60, 98.34)*			
1/b		IGRAb	7.3	58.12 (43.14, 86.23)	92.70 (77.84, 98.85)*	76.57 (48.06, 96.68)	NA	NA
		IGRAc	7.8	66.04 (46.97, 86.68)	96.72 (93.54, 98.68)*	65.37 (45.68, 91.79)	NA	NA
2/b		ELISA	8.4	53.85(44.41, 71.28)	92.42 (78.63, 98.76)*	83.79 (59.92, 97.78)	NA	NA

Model ‘a’: Two-dependent-test and one population model
Model ‘b’: One-test one population model
IGRAb: Interferon-gamma release assay using PPDb-PPDa antigens
IGRAc: Interferon-gamma release assay using peptide cocktail antigens
ELISA: Commercial Enzyme-immunosorbent assay
(*) Differences between the use of informative vs. uniform priors reflects a >10.5% variation in the posterior estimates

CHAPTER 5 - Modeling bTB within-herd dynamics with the use of different diagnostic strategies in high prevalence herds when depopulation is not feasible.

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5.1 Chapter summary

In Uruguay, bovine tuberculosis, a chronic disease of cattle, became endemic with high prevalence in large dairy herds, raising the concern of the authorities and the stakeholders, and threatening animal and public health. The lack of resources, together with the economic and social impact that slaughtering a complete herd imposes, makes depopulation an impractical alternative. The increase in bTB-prevalence was recently associated to demographic and management changes in the dairy industry in Uruguay challenging the current control program based on intradermal serial testing using the caudal fold- and comparative cervical- tuberculin test and slaughter of reactors. Here, we aimed at understanding the bTB-within-herd dynamics with the use of mathematical modeling. In order to assess the effectiveness of current and six alternative control scenarios that simultaneously minimized the slaughter of uninfected animals. We modified a compartmental age-structured frequency-dependent bTB-within-herd model parameterized and validated previously for Uruguay to simulate independently six alternative scenarios. The alternative control strategies assessed aimed to increase the sensitivity of detection and include: the single use of the caudal fold test (CFT) or the interferon gamma release assay (with the use of two different antigens-IGRAb and IGRAc-), and the use of parallel testing with CFT+IGRA, CFT and an Enzyme-linked immunosorbent assay (ELISA), or IGRA+ELISA every three months in adult animals (>1year). Results showed no significant differences in the time to reach bTB-eradication or official tuberculosis-free status (two consecutive negative test results) with any of the alternative strategies relative to the status quo, showing a consistent residual bTB-

infection for all the scenarios in the young categories (calves). Additionally the relative cost, assessed as the proportion of unnecessary slaughtered uninfected animals (false positives), significantly increased for all strategies in reference to the status quo. However, we demonstrate how the alternative strategies can significantly reduce bTB-prevalence when applied for restricted periods (6, 12 or 24 months), and in the case of IGRAc, without incurring higher unnecessary slaughter of animals in the first 6 months. The enhanced understanding of bTB-within-herd dynamics with the use of different control strategies helps to the advance in the identification of the optimal strategy for the control and eradication of bTB from dairy cattle in Uruguay and similar endemic settings.

5.2 Introduction

Mycobacterium bovis (*M. bovis*) is the main cause of bovine tuberculosis, one of the most widespread zoonotic bacterial infection that affects cattle and other mammals. Limited control success has been achieved worldwide (Bezos et al., 2014; Good et al., 2018; More, Radunz, & Glanville, 2015; Morris, 2015) in part because of the chronic nature of the disease, and the limited accuracy of the current diagnostic strategies developed (Schiller et al., 2010).

The use of bTB-intradermal testing has proven a useful tool for bTB-surveillance when detecting infected herds (Bezos et al., 2014; de la Rua-Domenech et al., 2006); however, the accuracy at the individual level is limited, in part because of host and pathogen characteristics (Gormley et al., 2006; Gormley et al., 2004), making challenging the eradication of disease when infection establishes. In herds with high prevalence, bTB-control using intradermal test-and-individual-slaughter is difficult; still, prompt control of the disease is crucial to avoid hazardous levels of pathogen circulation that can impose high zoonotic risk, and animal health and welfare impact.

To achieve control in high prevalence settings two main approaches have been used with variable success; a) the use of in-vitro tests as ancillary diagnostic strategy to improve the sensitivity for bTB detection (Council Directive 64/432/EEC, (Casal et al., 2014), and b) the depopulation of the complete herds in 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 animals infected with *M. bovis* that is likely missed by the intradermal testing. Whole herd depopulation, although costly, is an effective strategy to control and eradicate bTB (More et al., 2015). However, the complexity of its implementation increases with the size of the bTB-infected herd and is not always a feasible option. The lack of resources, together with the economic and social impact that culling a complete herd in which infection is confirmed in a low proportion of the animals involved, makes difficult to justify as a routine strategy for bTB-control programs in endemic settings (Ciaravino et al., 2017).

In Uruguay, the bTB-control program relies on serial intradermal testing, with the use of Caudal Fold Tuberculin test –CFT- as a screening test and the Comparative Cervical Tuberculin tests –CCT- for confirmation, followed by the slaughter of reactors and the bacteriological analysis. Herds in which bTB-infection is confirmed are subjected to intradermal retesting until two consecutive negative results in the whole herd are achieved to regain the officially tuberculosis-free status (OTF) (MGAP, 1989). Farmers with bTB-positive animals resulting in the slaughter are eligible for government indemnity (Law 19300, 26/12/2014.DGSG/MGAP).

The application of the national bTB-program in Uruguay was translated into a low herd prevalence (<0.001) for several years (WAHIS_OIE, 2014). However, in the past decade, we observed an increase in the number of bTB-infected dairy herds, the within-herd bTB-prevalence and the time from detection to the recovery of the officially

tuberculosis-free status (OTF) (Picasso-Risso et al., 2019), which led to unprecedented challenges in the control of bTB in Uruguay. This bTB-endemic scenario in Uruguay was associated with changes in dairies demographic structure and management, resulted from the consolidation of its industry (Picasso et al., 2017; Picasso-Risso et al., 2019). The observed variation of bTB dynamics followed the emergence of a dairy industry with larger herds (>360 animals) (DIEA, 2018), higher animal density, increased animal movements, and more intensive animal rearing than the traditional farming from the previous decades (Picasso et al., 2017). The current bTB-prevalence increase in the dairy industry raised the question whether the current bTB-program in Uruguay is efficient enough to control bTB in these dairy herds once the infection is confirmed and transmission is occurring.

Mathematical transmission models have been used broadly 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 allow accounting for the chronic nature of the disease, with long and variable incubation periods, biological variabilities, and different production systems (Alvarez, Bezos, et al., 2014), while avoiding the risks and the costs of implementing the control strategies in-vivo (Halasa & Dürr, 2017). For Uruguay, an integrated within-and between-herd model was parameterized and validated previously to evaluate the effect of risk-targeted bTB-surveillance with the use of the current test-and-slaughter bTB- program (VanderWaal et al., 2017). However, previous studies suggested that the sensitivity of the test-and-

slaughter program is impaired in high prevalence dairy herds in Uruguay (Picasso-Risso et al. *submitted*), and given depopulation of these large herds in Uruguay is not economically, logistically or socially feasible, the use of alternative diagnostic in-vitro assays, is a reasonable tool to reach control in these herds.

In this study, we simulated the application of the current and six mutually exclusive alternative bTB-control strategies, aiming to the assessment of which strategy is more effective to eradicate bTB from the herd, while minimizing the slaughter of uninfected animals, to ultimately, elucidate the optimal option for high prevalence dairy herds in Uruguay when depopulation is not an alternative.

5.3 Methods

Model description

Our interest was to use a mathematical transmission model that can capture and integrate three within-herd dynamics: herd demographics, bTB transmission, and control strategies in bTB-infected dairy herds with high prevalence (>10%) to evaluate the effectiveness of six alternative control scenarios relative to the status quo. We used a modified version of the stochastic, age-structured compartmental within-herd simulation model developed and parameterized previously for bTB patterns in Uruguayan cattle herds (VanderWaal et al., 2017). This model simulated the different dynamics in discrete monthly periods. The model was coded and run in R software (v3.2.4, R Foundation for Statistical Computing).

The outputs of the model were a) time to bTB-eradication, b) bTB-prevalence at the end of the first six months and each of the first ten years of simulations, c) time to regain the OTF status, and d) proportion of animals slaughtered (true and false positives).

Herd demographics

Demography parameters were modeled as previously described (VanderWaal et al., 2017). Briefly, we used two animal categories, adults (all animals >12 months), and calves (≤12 months), with calves moving into adult categories at a rate 1/12 months. Animal slaughter, births, and replacement occur on rates previously estimated for dairies in Uruguay (VanderWaal et al., 2017), following a Poisson distribution with an average proportion of non-infection related slaughters for adults (λsl.a) of 0.268, and for calves (λsl.c) of 0.007. In order to maintain stable herd size, births were assigned the same rate as λsl.c and replacement in the adult category the same rate as λsl.a every 4 months (frequency of s = 4). The model was initialized with an adult/calf ratio of 75/25 in a population of 500 animals following the typical replacement rates and demographic characteristics of large dairy herds in Uruguay (DIEA, 2018).

Individual-based bTB-transmission dynamics

bTB-infection was simulated using a stochastic, discrete, compartmental model, in which animals were assumed to transit four mutually exclusive stages; Susceptible (S), Occult (O), Reactors to diagnostic tests (R), and Infectious (I; SORI model) (Alvarez, Bezos, et al., 2014; G. Ciaravino et al., 2018; Conlan et al., 2012; Perez et al., 2002; VanderWaal et al., 2017). When healthy animals from the susceptible compartment ‘S’

are exposed to *M. bovis*, they move to the occult stage during a latent period (λ1) in which even though infected, they are not detected by any bTB-diagnostic test (antemortem test) and are not considered infectious. As disease progresses, these occult animals become reactors to the diagnostic tests at two different times of detection (λ2a and λ2b) corresponding to the sub-compartments Ra and Rb respectively: λ2a represents the period in which infected animals are only detected by the IGRA, and λ2b representing the time in which all tests can detect bTB-infected animals. The final compartment (I) represents animals that are infectious while also reactive to antemortem diagnostic testing (Figure 5.6.1). The same SORI-model was applied for the two age categories. The transition between compartments occurs following a Poisson process with rates based on the following deterministic backbone differential equations:

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

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

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

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

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

We assumed homogeneous mixing between adults and calves, and frequency-dependent transmission given that in dairies the likelihood of effective contact between

animals was assumed to remain independently of the size of the herds (Smith et al., 2013; VanderWaal et al., 2017).

At each time step, the number of animals that transitioned between each compartment was pulled from a Poisson distribution with the purpose of incorporating stochasticity to the model as was previously described (Gillespie, 2001; Keeling & Rohani, 2008).

Individual-based bTB-control dynamics

We evaluated six alternative control strategies scenarios (table 5.7.1). The alternative strategies were selected to improve the current sensitivity of the control program with the application of a maximum of two diagnostic tests at once. Testing was performed every three months to allow comparison with the status quo control strategy in Uruguay (MGAP, 1989).

We simulated the bTB-transmission dynamics independently with the application of each alternative strategy previously described. Each testing method had a specific beta distributed sensitivity –Se- and specificity –Sp- that related to the different stages of disease (SORI-compartments) (table 5.7.2) describe elsewhere (Picasso-Risso et al., submitted). Animals detected through the different diagnostic strategies were eliminated from the herd before the following testing period (i.e., every three months). We assume constant herd size during simulations, and we incorporated the same number of

slaughtered animals to the susceptible category in the following time step. We recorded the number of slaughtered animals using four new mutually exclusive compartments, one for the false positives (SS) and three for the true positives (RaS, RbS, IS) from each control strategy (Figure 5.6.1). The number of animals falling in each detected compartments were drawn from a Poisson distribution centered on the expected number of animals testing false positive in the susceptible (Ss), and true positive in the infected compartments (Ras, Rbs, Is) given by the equations 1 and 2 respectively for strategies involving one-test or parallel testing. Positive and negative correlation coefficients (rhoDc and rhoD) were included when combining two-diagnostic tests following the distributions described in the previous literature for Uruguay (Picasso-Risso et al. submitted).

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

$S_s = [1 - (Sp_1 * Sp_2 + rhoD)] * S_{ad}$ (parallel testing)

Eq 2. True Positives:

$Ra_s = Se * Ra_{ad} \quad Rb_c = Se * Rb_{ad} \quad I_s = Se * I_{ad}$ (single testing)

$Ra_s = (Se_1 * Se_2 + rhoDc) * Ra_{ad}$

$Rb_s = (Se_1 * Se_2 + rhoDc) * Rb_{ad}$

$I_s = (Se_1 * Se_2 + rhoDc) * I_{ad}$ (parallel testing)

Assessment of alternative strategies

To mimic the scenario of high bTB herd prevalence, we initially ran the model without the 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%, mimicking the worst scenario (i.e. highest bTB-prevalence) for dairy bTB-infected herds in Uruguay. Then, we used the median estimated number of animals in each of the infected compartments (O-R-I) to seed each of the six runs of the model evaluating the bTB-control strategies (Supplementary figure 5.8.S1). This was done in order to create a realistic distribution of animals across compartments in a high prevalence situation.

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

5.4 Results

Epidemiological indicators:

The median and 75ths, 95ths percentile estimates showed slightly different trends in the time to bTB-eradication in each scenario for the complete herd and categorized by age (figures 5.6.2-3). The median time to eradication ranged from 61 to 82 months, and

41 to 62 months if only adults are considered (table 5.7.3). Towards the end of the outbreak, calves carried most of the residual infections, which represent the category without bTB-control strategies applied, maintaining the circulation of the disease for significant (Kruskal-Wallis P-value <0.05) longer periods.

There were no significant differences in the overall bTB-prevalence distributions when comparing the six alternative scenarios relative to the status quo according to the Kruskal-Wallis test (P-value 0.59), and the log-rank test (P-value 0.29) (figure 5.6.4). However, when differences in the first six month and annual prevalence at the end of the first ten years of the simulations were assessed, we detected significant differences in the first three periods (6,12,24 months) (P-value<0.05), and no differences in the subsequent ones. Status quo prevalence at six month (1.02% 95th%ile: 8.4-11.8) and the first year (5.7%, 95th %ile: 4.6-7.6statistically differ from the prevalence estimates for the other six alternative scenarios (table 5.7. 4, figure 5.8.S2-S3) (P-values <0.05). At the end of the second year of simulations, the bTB-prevalence distributions of the status quo (2.5%, 95th %ile: 1.2-5.2) was significantly different from the CFT+IGRA (1.8%, 95th %ile: 1.1-3.6) (P-value 0.01), and in the margin of significance with the IGRAb (2.2%, 95th %ile: 1.0-3.8), and IGRA+ELISA (2.4%, 95th %ile: 0.8-4.2) (P-value<0.1) (table 5.7.4, figure 5.8.S4).

Time to regain the OTF status did not vary between the strategies simulated according to the Kruskal-Wallis test, or the log-rank test (P-value >0.05) (figure 5.6.5), with median estimates ranging between 50 and 59 months (4.1 to 4.9 years).

Performance effectiveness

The simulated scenarios using ELISA as an ancillary test (IGRA+ELISA and CFT+ELISA) have a larger proportion of animals testing positive (figure 5.6.6), with larger rates of false positives (15.47%, 95th %ile:12-19.1 and 17.73%, 95th %ile: 14.1-21.2 (figure 5.6.7), than the status quo or the other alternative scenarios. The status quo scenario has the lowest proportion of positive diagnostic results, and false positive results (median: 0.8% 95th %ile: 0.5-1.3), which distribution was significantly different from all the other simulated strategies according to the Kruskal-Wallis test.

5.5 Discussion

In Uruguay, the bTB-control program in dairies is based on a strategy that provides high diagnostic specificity (CFT and CCT serial testing) to avoid unnecessary animal culling (false positives), which resulted in historical low prevalence values reported within- and between- herds until the past decade (WAHIS_OIE, 2014). Since then, a consistent increase in the reported number of bTB-infected dairy herds and its within-herd prevalence in the past decade associated to demographic and management changes in the industry led to challenging the effectiveness of the current bTB-control program in this evolving population.

In this study, we intended to understand the effectiveness of alternative control strategies, directed to the increase in the bTB-diagnostic sensitivity (with single or parallel testing), on the bTB-within-herd dynamics in highly prevalent dairy herds in Uruguay, while minimizing the culling of uninfected animals. To do so, we utilized

mathematical models that can help us to embrace the uncertainty associated with the long duration of the disease, the lack of clinical signs, and variability in disease dynamics and transmission patterns (Brooks-Pollock et al., 2014; G. Ciaravino et al., 2018). With mathematical modeling, we benefit from the avoidance of risk and costs associated with field implementation of the different strategies and can help decision-making (Alvarez, Bezos, et al., 2014; Perez et al., 2002).

Here, we initially simulated bTB-transmission in 500-size dairy herd (>75percentile for dairies in Uruguay) (DIEA, 2018; Picasso et al., 2017), with two categories of animals (adults and calves), and an apparent prevalence of 10% to subsequently simulate a conservative scenario towards eradication, with the application of seven control strategies (status quo and six alternatives) every three months. We intended to simulate the current demographic and endemic characteristics of the most challenging bTB-dairy outbreaks currently active in Uruguay, and the most conservative testing scenario to improve the detection sensitivity.

An advantage of the IGRAs involved the marginal earlier detection (~2 weeks) of the bTB- cell-mediated immune response in comparison to the intradermal test or the ELISA assay (Bezos, Casal, et al., 2014; de la Rua-Domenech et al., 2006). The inclusion of two reactors subcompartments (Ra and Rb) was important to account for the variations in duration of the detection period for the IGRAs and the intradermal and ELISA assay.

Our findings indicated that there were no significant differences overall to reach bTB-eradication or OTF-status with the six strategies relative to the status quo (73

months for status quo strategy, 95_{th} %ile: 36-103, and 59 months for alternative strategies, 95_{th} %ile: 26-122 months respectively); and the relative costs associated with slaughtering uninfected animals will be significantly incremented with the use of alternative strategies, with the worse estimated performance observed with strategies that included the ELISA (table 5.7.3). The lack of significant improvement can be associated with the maintenance of the disease in the calves' category (figure 5.6.3). In most of the simulated scenarios, eradication is reached earlier in the adult category than the calves (table 5.7.3), who remain undetected until reaching the opportunity to be tested when they reach >12 months old, and responsible in most of the simulations of sustaining bTB in the herd for longer periods (table 5.7.3, figure 5.6.3). Thus, we need to consider that this conclusion might not hold when simulating control strategies that include calfdhood testing.

When exploring the effect of the control strategies in shorter periods, after 6, 12 and 24 months, we interestingly observed a significant reduction in bTB-prevalence at the end of 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. These results demonstrate that alternative strategies can be selected as an initial strategy, with the following use of the current status quo for eradication, when following the assumptions of the model. In addition, when the strategies were assessed for unnecessary slaughter, the IGRAc, matched the performance of the status quo in the first 6 months of testing (P-value >0.05) (supplementary figure 5.8.S4), suggesting that might be an effective tool to

reduce bTB-prevalence at initial stages of the control program (six months or two consecutive tests).

In the different control scenarios, the sensitivity and specificity of the diagnostic tools applied were based on estimations for accuracy for high bTB-prevalence dairy herds in Uruguay (Picasso-Risso et al., *submitted*), which can help to reduce the ambiguity (and sometime contradictory) test performance (Alvarez, Bezos, et al., 2014; G. Ciaravino et al., 2018). Testing intervals (3 months) represent the highest possible pressure for detection for intradermal testing in which we can elude the anergy period (de la Rua-Domenech et al., 2006; Radunz & Lepper, 1985; Vordemeier et al., 2006), and slaughter is logistically possible before next testing period. Although in-vitro testing allows more frequent testing, and can benefit from the booster effect after tuberculin inoculation when applied in parallel (CFT+IGRA) (Casal et al., 2014; M V Palmer et al., 2006; Irene Schiller et al., 2010), we prefer to assess the strategies in reference to the status quo, and avoided the inclusion of shorter testing-intervals. Nevertheless, a deeper understanding of the effect of different testing periods is needed, before the elaboration of recommendations for decision-making.

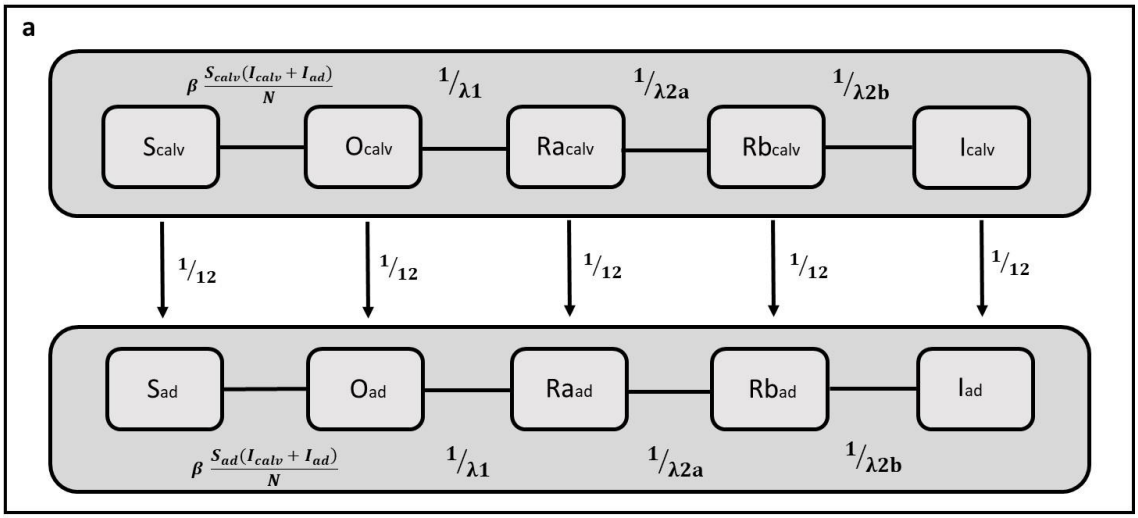
In order to evaluate the optimal strategies, we balanced the epidemiological effectiveness of the control while minimizing unnecessary culling of false reactors relative to the status quo. While an initial approximation of the additional efforts that will impose each strategy is still essential to estimate the economic cost (Kao, Roberts, &

Ryan, 1997; Kao et al., 2018; Smith et al., 2013) and social acceptance (Ciaravino et al., 2017) before its implementation.

Here, we conclude that alternative strategies assessed do not improve the time to bTB-control or reduce the false positive slaughters overall, but most importantly we increased the understanding of bTB-dynamics in adult and calf categories when applying different testing pressures in these highly infected dairy herds in Uruguay. Additionally, we showed the importance of target control strategies to infected calves, the potential benefit of using the IGRAc in the initial stages of the control when bTB-prevalence is ~10%, without incurring in additional unnecessarily slaughters, and the poor control reached with the ELISA. The determination of the best strategy will be a result of epidemiological, performance and economic balance while acknowledging the country logistics and socio-cultural perceptions, and with our results here, we enhance the understanding of bTB-within-herd dynamics that reduce the gap in the knowledge for identification of the optimal bTB-control strategy for dairies in Uruguay and similar endemic settings.

5.6 Figures

Figure 8: Diagram representing the bTB-transmission flow between compartments (figure 1a) including calves (top row) and adults (second row), and control strategies dynamics (figure 1b). Number of animals in each bTB-compartment are indicated as susceptible (S), occult (O), reactors in subgroup a (Ra) and b (Rb), and infectious (I). Transmission rates between infectious and susceptible stage are represented by β, and the duration of the occult, reactors a, and reactors b stages are represented by λ1, λ2a, λ2b. The equations for the probability of testing positive to the control strategies included sensitivity (Se), specificity (Sp), and correlation coefficients between negative (rhoD) and positive (rhoDc) results respectively.


a
$\beta \frac{S_{calv}(I_{calv}+I_{ad})}{N}$
$^1/_{\lambda 1}$
$^1/_{\lambda 2a}$
$^1/_{\lambda 2b}$
Scalv
Ocalv
Racalv
Rbcalv
Icalv
$^1/_{12}$
$^1/_{12}$
$^1/_{12}$
$^1/_{12}$
$^1/_{12}$
Sad
Oad
Raad
Rbad
Iad
$\beta \frac{S_{ad}(I_{calv}+I_{ad})}{N}$
$^1/_{\lambda 1}$
$^1/_{\lambda 2a}$
$^1/_{\lambda 2b}$

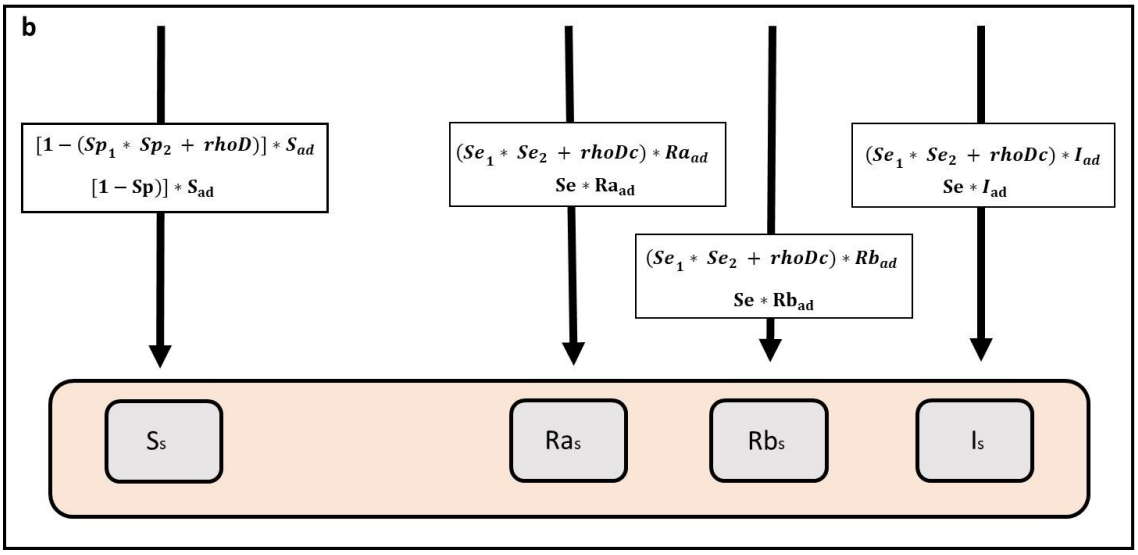
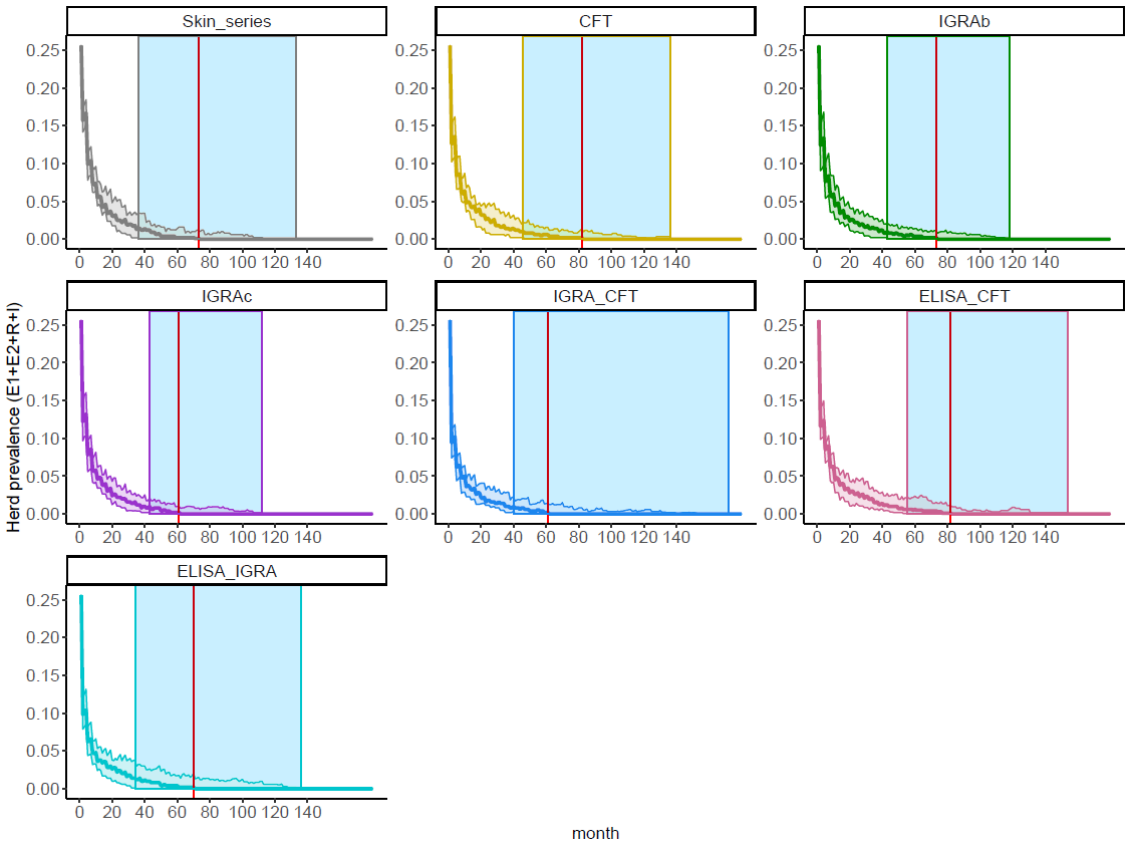

b
$[1-(Sp_1 * Sp_2 + rhoD)] * S_{ad}$
$[1-\mathrm{Sp})] * \mathrm{S_{ad}}$
$(Se_1 * Se_2 + rhoDc) * Ra_{ad}$
$\mathrm{Se} * \mathrm{Ra_{ad}}$
$(Se_1 * Se_2 + rhoDc) * Rb_{ad}$
$\mathrm{Se} * \mathrm{Rb_{ad}}$
$(Se_1 * Se_2 + rhoDc) * I_{ad}$
$\mathrm{Se} * I_{ad}$
Ss
Ras
Rbs
Is

Figure 9: Median, five and 95$_{th}$ 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.



CFT: Caudal Fold tuberculin test
Skin_series: CFT and Comparative Cervical tuberculin test serial testing
IGRAb: Interferon-gamma release assay using PPDb-PPDa antigens
IGRAc: Interferon-gamma release assay using peptide cocktail antigens
ELISA: Commercial Enzyme-immunosorbent assay

Figure 10: Median, and 95th 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.

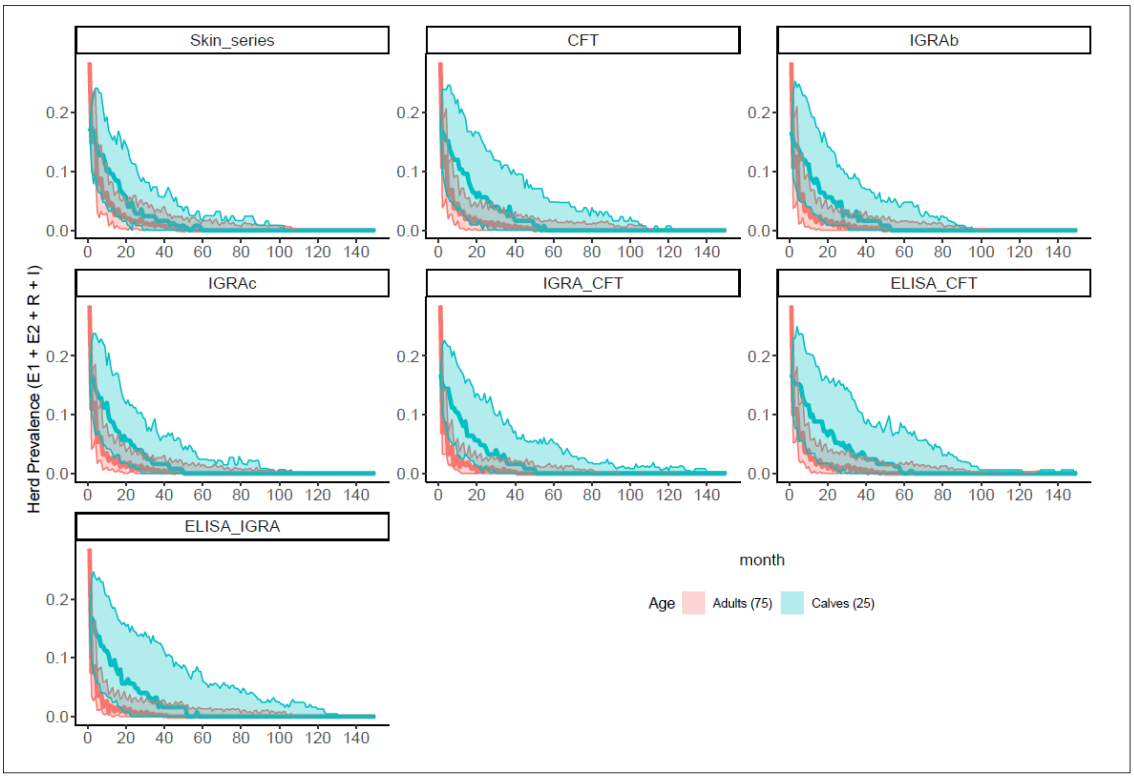


Figure 11: Comparison of time to eradication for status quo and alternative control strategies. Boxplot (right) shows the number of months to reach prevalence zero per strategy (median, interquartile range, and 95$_{th}$ percentile whiskers). Survival curve shows the median time to reach eradication. No significant differences were observed with the Kruskal-Wallis or the log-rank tests.

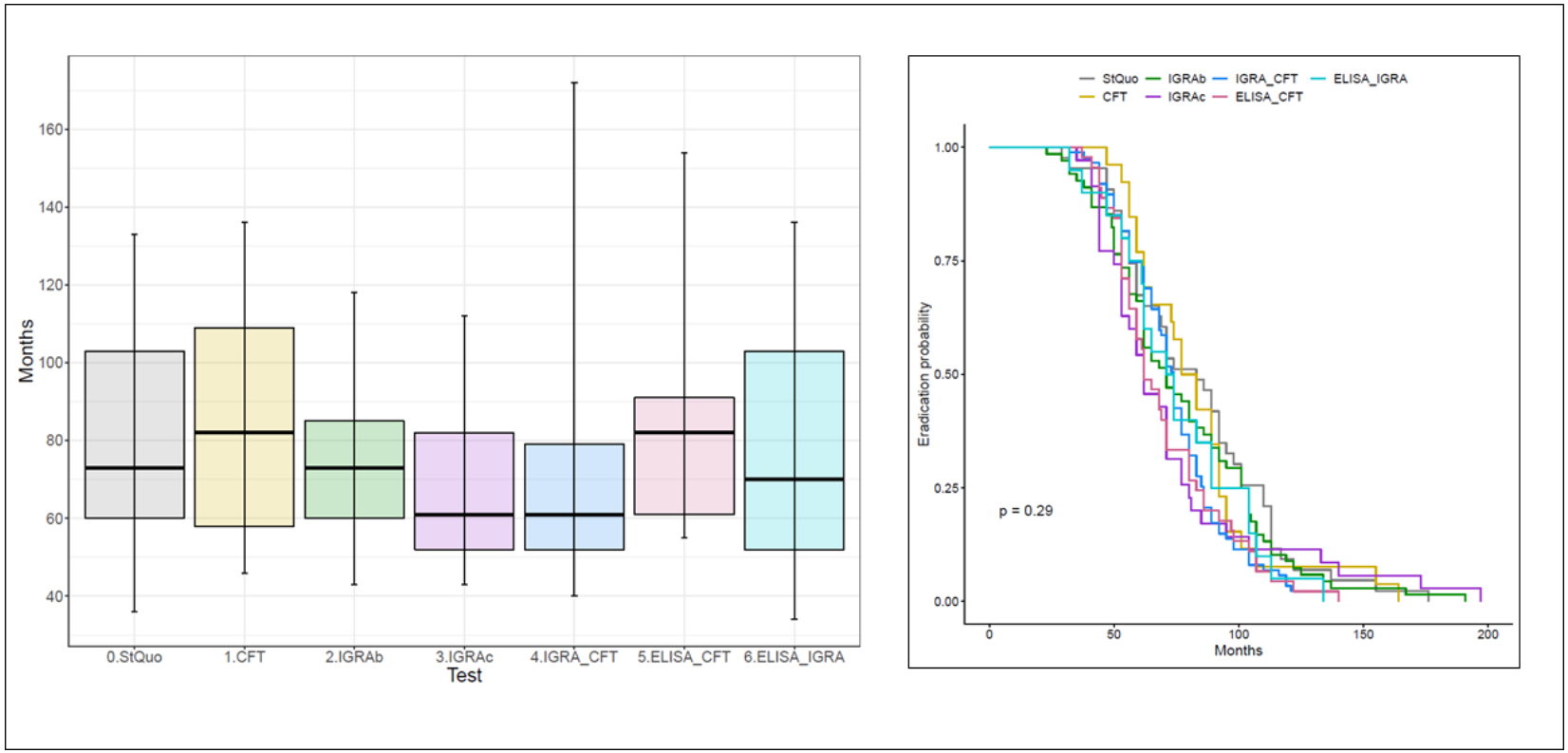


Figure 12: Comparison of time to reach the officially tuberculosis-free status for the 500 simulations with the status quo and six alternative control strategies. Boxplot (median, interquartile-range and 95$_{th}$ percentile whiskers) shows the number of months to reach two consecutive true negative bTB-diagnostic results per strategy.

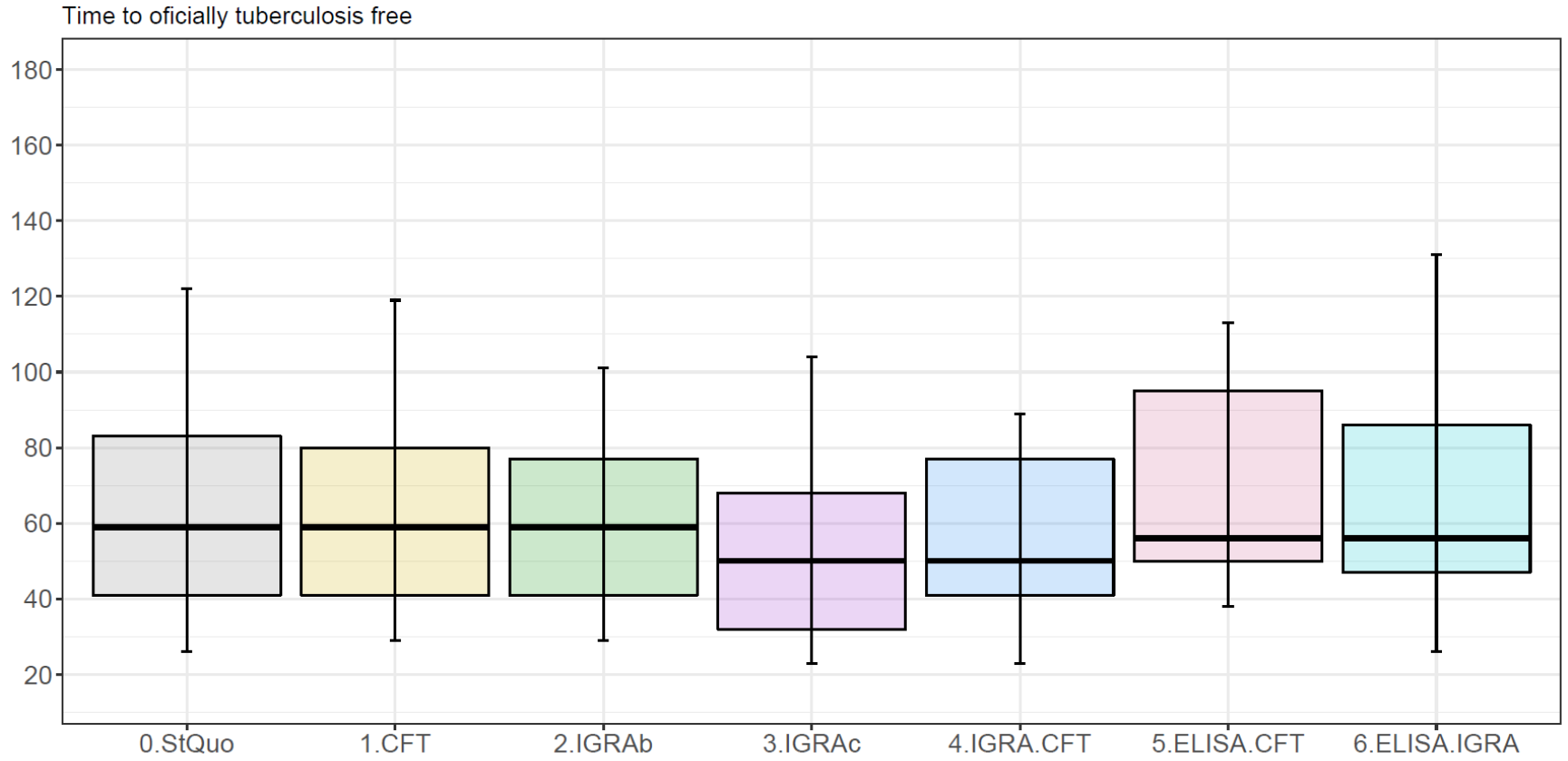


Figure 13: Median and 95_{th} percentile of the proportion of animals testing positive (pink), and testing true positive (turquoise) the status quo (0.Skin_series) and the six alternative control scenario per month.

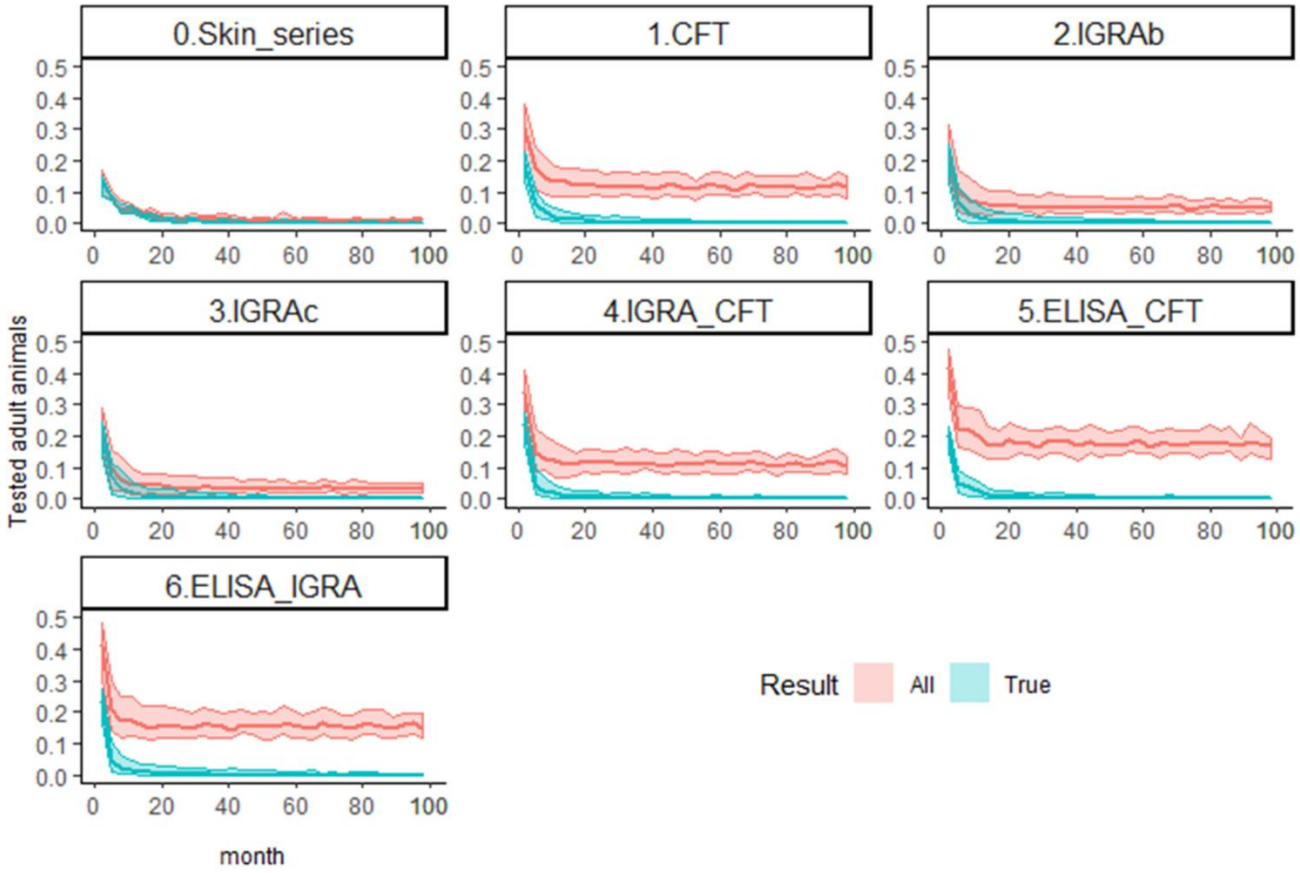
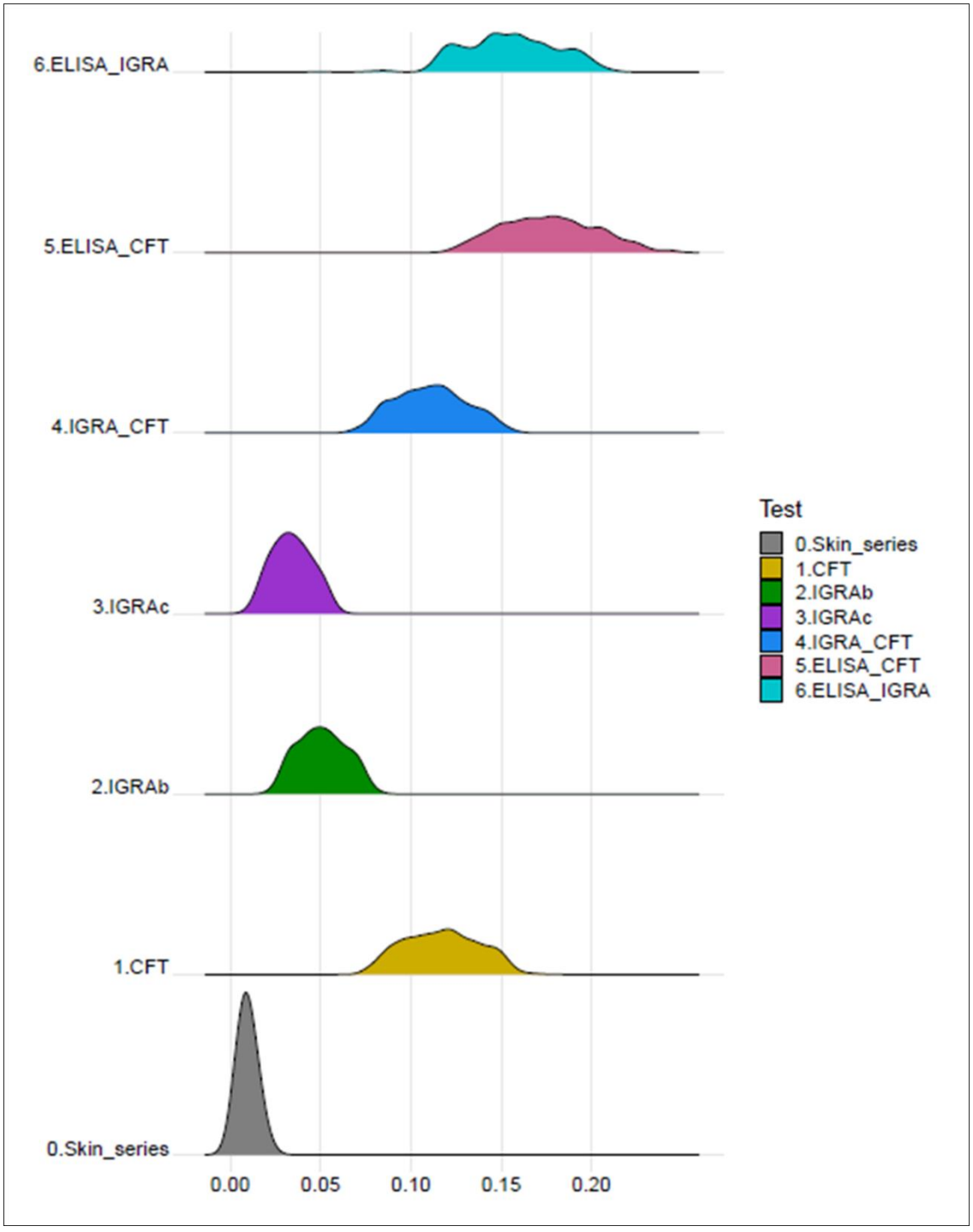


Figure 14: Distribution (median, 95_{th} percentiles) of the 500 simulations of the proportion of animals testing false positive over the total adults tested, to the status quo (0.Skin_series) and the six alternative control scenario per testing implemented.



5.7 Tables

Table 9: Control strategies evaluated by the model

Strategy	Combination	Test 1	Test2
Status Quo	Serial	CFT	CCT
1. CFT	NA	CFT	NA
2. IGRAb	NA	IGRAb	NA
3. IGRAc	NA	IGRAc	NA
4. CFT + IGRA	Parallel	CFT	IGRA
5. CFT + ELISA	Parallel	CFT	ELISA
6. IGRA + ELISA	Parallel	IGRAc	ELISA

CFT: Caudal Fold tuberculin test
CCT: Comparative Cervical tuberculin test
IGRAb: Interferon-gamma release assay using PPDb-PPDa antigens
IGRAc: Interferon-gamma release assay using peptide cocktail antigens
ELISA: Commercial Enzyme-immunosorbent assay

Table 10: Sensitivity and specificity estimates used for modeling each bTB-testing strategy.

Strategy	Sensitivity*	dβeta (α_1, α_2)	Specificity*	dβeta (α_1, α_2)
Status Quo	53.27 (45.76, 60.59)	89.1, 78.6	96.56 (93.34, 98.52)	60.4, 1.6
CFT	73.34 (56.88,89.44)	18.6, 6.8	77.02 (58.96, 95.48)	23.5, 3.8
IGRAb	78.01 (62.97, 89.53)	26.7, 7.9	91.43 (78.91, 98.26)	27.8, 3.2
IGRAc	76.21 (65.35, 85.86)	46.3, 14.4	96.56 (93.34, 98.52)	324.9, 13.5
ELISA	53.85(44.41, 71.28)	83.7, 76.1	92.42 (78.63, 98.76)	26.4, 2.7

CFT: Caudal Fold tuberculin test
CCT: Comparative Cervical tuberculin test
IGRAb: Interferon-gamma release assay using PPDb-PPDa antigens
IGRAc: Interferon-gamma release assay using peptide cocktail antigens
ELISA: Commercial Enzyme-immunosorbent assay
(*) Median and 95PPI estimates obtained from Picasso-Risso et al. (submitted)

Table 11: 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 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 for the adult animals solely. 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.

		Complete herd (eradication)					Adults (eradication)					OTF				
Test		q2.5	q25	med	q75	q97.5	q2.5	q25	med	q75	q97.5	q2.5	q25	med	q75	q97.5
StQuo	Years	3.0	5.0	6.1	8.6	11.1	2.6	3.8	4.3	8.3	11.1	2.2	3.4	4.9	6.9	10.2
	Months	36	60	73	103	133	31	46	52	100	133	26	41	59	83	122
	Tests	13	21	25	35	45	11	16	18	34	45	9	14	20	28	41
CFT	Years	3.8	4.8	6.8	9.1	11.3	1.8	3.6	4.3	7.6	9.3	2.4	3.4	4.9	6.7	9.9
	Months	46	58	82	109	136	22	43	52	91	112	29	41	59	80	119
	Tests	16	20	28	37	46	8	15	18	31	38	10	14	20	27	40
IGRAb	Years	3.6	5.0	6.1	7.1	9.8	1.3	2.7	4.3	5.6	8.6	2.4	3.4	4.9	6.4	8.4
	Months	43	60	73	85	118	16	32	52	67	103	29	41	59	77	101
	Tests	15	21	25	29	40	6	11	18	23	35	10	14	20	26	34
IGRAc	Years	3.6	4.3	5.1	6.8	9.3	1.3	2.3	3.6	5.1	8.8	1.9	2.7	4.2	5.7	8.7
	Months	43	52	61	82	112	16	28	43	61	106	23	32	50	68	104
	Tests	15	18	21	28	38	6	10	15	21	36	8	11	17	23	35
CFT_IGRA	Years	3.3	4.3	5.1	6.6	14.3	0.9	2.7	3.3	5.8	13.1	1.9	3.4	4.2	6.4	7.4
	Months	40	52	61	79	172	11	32	40	70	157	23	41	50	77	89
	Tests	14	18	21	27	58	4	11	14	24	53	8	14	17	26	30
CFT_ELISA	Years	4.6	5.1	6.8	7.6	12.8	2.6	2.8	4.1	7.3	10.8	3.2	4.2	4.7	7.9	9.4
	Months	55	61	82	91	154	31	34	49	88	130	38	50	56	95	113
	Tests	19	21	28	31	52	11	12	17	30	44	13	17	19	31	38
IGRA_ELISA	Years	2.8	4.3	5.8	8.6	11.3	1.3	2.1	3.4	6.0	10.3	2.2	3.9	4.7	7.2	10.9
	Months	34	52	70	103	136	16	25	41	72	124	26	47	56	86	131
	Tests	12	18	24	35	46	6	9	14	25	42	9	16	19	29	44

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

Diagnostic test	month	bTB-prevalence		
		median	5_{th} percentile	95_{th} percentile
Skin series (Status quo)	6	0.102	0.084	0.118
	12	0.057	0.046	0.076
	24	0.025	0.012	0.052
CFT	6	**0.086**	**0.066**	**0.110**
	12	**0.050**	**0.038**	**0.072**
	24	0.028	0.008	0.046
IGRAb	6	**0.080**	**0.056**	**0.107**
	12	**0.046**	**0.027**	**0.071**
	24	0.022	0.010	0.039
IGRAc	6	**0.082**	**0.058**	**0.100**
	12	**0.048**	**0.034**	**0.064**
	24	0.022	0.010	0.038
IGRA_CFT	6	**0.064**	**0.046**	**0.079**
	12	**0.038**	**0.023**	**0.052**
	24	**0.018**	**0.011**	**0.036**
ELISA_CFT	6	**0.088**	**0.070**	**0.098**
	12	**0.048**	**0.029**	**0.062**
	24	0.026	0.014	0.048
ELISA_IGRA	6	**0.064**	**0.045**	**0.085**
	12	**0.038**	**0.026**	**0.055**
	24	0.024	0.008	0.042

CFT: Caudal Fold tuberculin test
Skin_series: CFT and Comparative Cervical tuberculin test serial testing
IGRAb: Interferon-gamma release assay using PPDb-PPDa antigens
IGRAc: Interferon-gamma release assay using peptide cocktail antigens
ELISA: Commercial Enzyme-immunosorbent assay
Kruskal-Wallis significant different prevalence estimates (P-value <0.05) relative to the status quo are represented in bold

CHAPTER 6 – General discussion and conclusions

This research provides the foundations for a better understanding of the bTB-epidemiology and diagnostic tools and their impact in bTB-within herd dynamics when applied in the control of bTB in endemic settings. In addition, results here approach the existing gap in the elucidation of the optimum control strategies to be implemented for bTB-eradication.

With the assessment of the diagnostic interaction between JD and bTB in the second and third chapter of this dissertation, we demonstrated that positive JD and bTB animals tend to coexist in the herd. Because the limited number of bTB-infected herds in Uruguay could represent a limitation for the robustness of the herd level analyses, we conducted the study in Castilla y Leon (CyL), Spain. CyL is a bTB-endemic region, that suited the characteristics needed for this study (bTB-prevalence levels >1%, test-and-slaughter bTB-control program, and the presence of JD in the cattle herds), making it an optimum setting to address this goal. Moreover, variations in the extent of bTB and JD interaction patterns depended on both diseases prevalence levels. Among bTB-high prevalence and JD- coinfected herds (Spain), the JD-antibody response is more likely to occur when frequent inoculations of tuberculin are used, while in JD- bTB- high prevalence coinfected herds (Uruguay) the cell-mediated immune response tend to occur in higher proportions in animals with specific JD-antibodies. Results here may indicate that animals infected with one of the diseases would develop a cross-reactive immune response to the other disease, increasing the sensitivity of the other test or reducing its specificity. Alternatively, a similar effect can be inferred from those animals that are frequently inoculated with the intradermal tuberculin test, but not bTB-positives. While not concluding in

elucidating the interaction between true disease statuses of the animals, these findings highlighted the need to adjust the performance estimates for bTB-diagnosis in bTB- and JD- coinfected herds.

Consequently, in chapter four, we assessed the performance of bTB-in-vitro tools, with potential use in the high bTB-JD-prevalence endemic populations in Uruguay. With this study, we confirmed the improvement in the sensitivity of diagnostic that can be achieved with the use of IGRA, and the potential applicability of ELISA as an ancillary tool to increase the specificity, expanding the understanding of bTB-in-vitro tools performance in heavily bTB- and JD-coinfected herds and Uruguay.

Finally, in chapter five we integrate the knowledge acquired in the previous chapters with simulation models to understand the effect in bTB-within-herd dynamics, and epidemiological and performance effectiveness of six alternative control scenarios that simultaneously minimized the slaughter of uninfected animals. With the evaluation of alternatives that improved the sensitivity of detection (single testing or combinations in parallel), we demonstrated that variations in within-herd dynamics were not substantial to improve bTB- control, -time-to-eradication or minimize the unnecessary slaughter.

As a whole, this research contributes to approaching the gap in knowledge of the potential effect of the use of in-vitro and intradermal testing strategies as part of a differential control program in high-prevalence bTB- and JD- coinfected herds, enhancing the understanding that will be the foundation of the optimal bTB-control strategy in endemic settings, when depopulation is not suitable. The incorporation of field estimates for chronically and heavily bTB-infected herds, and demographic

vicissitudes represents a novelty in the assessment of bTB-control strategies for high prevalence bTB-endemic areas, and it is the first attempt to address JD- and bTB- co-infection for bTB-control. In addition, it exposes the need to explore the epidemiological advantages and disadvantages of the implementation of bTB and JD integrated control and eradication programs in highly coinfected populations.

While addressing the primary goal of this research, we disregard the true bTB- and JD- animal status of the animals when assessing diagnostic interference and accuracy, which limits the understanding on the extent of the impact of one disease on the other. However, the scientific consensus on the lack of a sensitive gold standard method supports the selection of Bayesian statistics and modeling analytical tools to embrace this uncertainty and generate valid results.

As scientific based research, as the more we learn, the more hypothesis and questions we generate. While we accomplished the goal of this dissertation, more questions have raised that could be addressed in the future such as:

- What would be the most cost-benefit strategy for bTB-eradication in coinfected endemic settings?
- What are the individual, or pathogen (e.g., strains) factors increasing the susceptibility (or resistance) for bTB and JD coinfection here?
- What is the effect of other coinfections (e.g., viruses, parasites) in the accuracy of bTB-diagnostics tools and effectiveness of bTB-control?
- Are these findings valid to other demographic and management patterns?

- In these heavily bTB-infected settings, there is a higher risk for disease transmission due to alternative routes, often disregarded, that will affect the force of infection estimate assumed?

- What is the potential zoonotic impact of aiming bTB eradication in high-prevalence herds with the use of test-and-slaughter strategies instead of depopulation?

The epidemiology and diagnosis of bTB are very complex, intriguing and ambiguous. Although, significant advances have been made in understanding and addressing the limitations of bTB-diagnosis and eradication constraints, the continuous evolution of the animal production systems, the economy, and the socio-cultural trends, demands a continuous assessment and readjustment of bTB-control strategies. As an animal-health scientific community, we have the responsibility to reach a bTB-epidemiological understanding to reduce its burden in animals, people and the environment. The WHO “end-TB strategy” creates the unique opportunity to reunite forces and reach bTB-eradication soon. This dissertation intends to be a grain of sand in work to achieve that goal, which hopefully, will encourage others in the passion of fighting against this ancient challenge.

REFERENCES

64/432/EEC. (1964). Council directive on animal health problems affecting intra-Community trade in bovine animals and swine.

Aagaard, C., Govaerts, M., Meikle, V., Vallecillo, A. J., Gutierrez-Pabello, J. A., Suarez-Güemes, F., … Pollock, J. M. (2006). Optimizing Antigen Cocktails for Detection of Mycobacterium bovis in Herds with Different Prevalences of Bovine Tuberculosis: ESAT6-CFP10 Mixture Shows Optimal Sensitivity and Specificity. Journal of Clinical Microbiology, 44(12), 4326. https://doi.org/10.1128/JCM.01184-06

Abdallah, A. M., Gey van Pittius, N. C., DiGiuseppe Champion, P. A., Cox, J., Luirink, J., Vandenbroucke-Grauls, C. M. J. E., … Bitter, W. (2007). Type VII secretion — mycobacteria show the way. Nature Reviews Microbiology, 5, 883.

Al-Mouqatea, S., Alkhamis, M., Akbar, B., Ali, A., Al-Aqeel, H., Bin-Heji, A., … Perez, A. (2018). Bayesian estimation of ELISA and gamma interferon test accuracy for the detection of bovine tuberculosis in caudal fold test–negative dairy cattle in Kuwait. Journal of Veterinary Diagnostic Investigation, 30(3), 468–470. https://doi.org/10.1177/1040638718759574

Alvarez, J., Bezos, J., de la Cruz, M. L., Casal, C., Romero, B., Domínguez, L., … Pérez, A. (2014). Bovine tuberculosis: Within-herd transmission models to support and direct the decision-making process. Bovine Tuberculosis, 97, S61–S68. https://doi.org/10.1016/j.rvsc.2014.04.009

Alvarez, J., de Juan, L., Bezos, J., Romero, B., Sáez, J. L., Gordejo, F. J. R., … Aranaz, A. (2008). Interference of paratuberculosis with the diagnosis of tuberculosis in a goat flock with a natural mixed infection. Veterinary Microbiology, 128(1–2), 72–80. https://doi.org/10.1016/j.vetmic.2007.08.034

Alvarez, J., de Juan, L., Bezos, J., Romero, B., Sáez, J. L., Marqués, S., … Aranaz, A. (2009). Effect of paratuberculosis on the diagnosis of bovine tuberculosis in a cattle herd with a mixed infection using interferon-gamma detection assay. Veterinary Microbiology, 135(3–4), 389–393. https://doi.org/10.1016/j.vetmic.2008.09.060

Alvarez, J., Perez, A., Bezos, J., Marqués, S., Grau, A., Saez, J. L., … Domínguez, L. (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. https://doi.org/10.1016/j.vetmic.2011.07.034

Alvarez, J., Perez, A., Marqués, S., Bezos, J., Grau, A., de la Cruz, M. L., … Domínguez, L. (2014). Risk factors associated with negative in-vivo diagnostic results in bovine tuberculosis-infected cattle in Spain. BMC Veterinary Research, 10, 14–14. https://doi.org/10.1186/1746-6148-10-14

Aranaz, A. (1999, August 30). Mycobacterium tuberculosis subsp caprae subsp nov.: A Taxonomic Study of a New Member of the Mycobacterium tuberculosis Complex Isolated from Goats in Spain. Tuberculosis & Airborne Disease Weekly. Retrieved from http://link.galegroup.com/apps/doc/A55604272/EAIM?u=umn_wilson&sid=EAIM&xid=4ebe4bd3

Aranaz, Alicia, Cousins, D., Mateos, A., & Dominguez, L. (2003). Elevation of Mycobacterium tuberculosis subsp. caprae Aranaz et al. 1999 to species rank as Mycobacterium caprae comb. nov., sp. nov. International Journal of Systematic and Evolutionary Microbiology, 53(Pt 6), 1785–1789. https://doi.org/10.1099/ijs.0.02532-0

Aranaz, Alicia, De Juan, L., Montero, N., Sanchez, C., Galka, M., Delso, C., … Dominguez, L. (2004). Bovine tuberculosis (Mycobacterium bovis) in wildlife in Spain. Journal of Clinical Microbiology, 42(6), 2602–2608. https://doi.org/10.1128/JCM.42.6.2602-2608.2004

Aranaz, Alicia, juan, L. D., Bezos, J., Álvarez, J., Romero, B., Lozano, F., … Domínguez, L. (2006). Assessment of diagnostic tools for eradication of bovine tuberculosis in cattle co-infected with Mycobacterium bovis and M. avium subsp. paratuberculosis. Vet. Res., 37(4), 593–606.

Ayele, W., Machackova, M., & Pavlik, I. (2001). The transmission and impact of paratuberculosis infection in domestic and wild ruminants. Veterinarni Medicina, 46(7–8), 205–224. https://doi.org/10.17221/7878-VETMED

Baldwin, C. L., & Telfer, J. C. (2015). The bovine model for elucidating the role of γδ T cells in controlling infectious diseases of importance to cattle and humans. Non-Rodent Animal Models for Immunology Research, 66(1), 35–47. https://doi.org/10.1016/j.molimm.2014.10.024

Bang, B. (1908). Measures taken against animal tuberculosis in Denmark. Comparative Pathology and Therapeutics, XXI(4). Retrieved from Proceedings International Congress on Tuberculosis, Washington DC.

Barandiaran, S., Martínez Vivot, M., Pérez, A. M., Cataldi, A. A., & Zumárraga, M. J. (2015). Bovine tuberculosis in domestic pigs: Genotyping and distribution of isolates in Argentina. Research in Veterinary Science, 103, 44–50. https://doi.org/10.1016/j.rvsc.2015.09.013

Barbara Dufour, Régis Pouillot, & Benoît Durand. (2004). A cost/benefit study of paratuberculosis certification in French cattle herds. Vet. Res., 35(1), 69–81. https://doi.org/10.1051/vetres:2003045

Bezos, J., Álvarez, J., Romero, B., de Juan, L., & Domínguez, L. (2014). Bovine tuberculosis: Historical perspective. Bovine Tuberculosis, 97, Supplement, S3–S4. https://doi.org/10.1016/j.rvsc.2014.09.003

Bezos, J., Casal, C., Romero, B., Schroeder, B., Hardegger, R., Raeber, A. J., … Domínguez, L. (2014). Current ante-mortem techniques for diagnosis of bovine tuberculosis. Bovine Tuberculosis, 97, Supplement, S44–S52. https://doi.org/10.1016/j.rvsc.2014.04.002

Branscum, A., Gardner, I. A., & Johnson, W. (2005). Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling (Vol. 68). https://doi.org/10.1016/j.prevetmed.2004.12.005

BRAUN, H., & LEBEK, G. (1958). [Incidence of bovine tuberculosis infection in humans in Upper Bavaria and Swabia and the biology of both types of mammalian tubercle bacteria]. Medizinische Monatsschrift, 12(1), 33–39.

Brito, B. P., Aly, S. S., Anderson, R. J., Fossler, C. P., Garry, F. B., & Gardner, I. A. (2014). Association between caudal fold tuberculin test responses and results of an ELISA for Mycobacterium avium subsp paratuberculosis and mycobacterial culture of feces in tuberculosis-free dairy herds. Journal of the

American Veterinary Medical Association, 244(5), 582–587. https://doi.org/10.2460/javma.244.5.582

Brooks, S. P., & Gelman, A. (1998). General Methods for Monitoring Convergence of Iterative Simulations. Journal of Computational and Graphical Statistics, 7(4), 434–455. https://doi.org/10.2307/1390675

Brooks-Pollock, E., Roberts, G. O., & Keeling, M. J. (2014). A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature, 511(7508), 228–231.

Buzdugan, S. N., Chambers, M. A., Delahay, R. J., & Drewe, J. A. (2016). Diagnosis of tuberculosis in groups of badgers: an exploration of the impact of trapping efficiency, infection prevalence and the use of multiple tests. Epidemiology and Infection, 144(8), 1717–1727. https://doi.org/10.1017/S0950268815003210

Byrne, A. W., Kenny, K., Fogarty, U., O'Keeffe, J. J., More, S. J., McGrath, G., … Dohoo, I. R. (2015). Spatial and temporal analyses of metrics of tuberculosis infection in badgers (Meles meles) from the Republic of Ireland: Trends in apparent prevalence. Preventive Veterinary Medicine, 122(3), 345–354. https://doi.org/10.1016/j.prevetmed.2015.10.013

Casal, C., Bezos, J., Díez-Guerrier, A., Álvarez, J., Romero, B., de Juan, L., … Aranaz, A. (2012). Evaluation of two cocktails containing ESAT-6, CFP-10 and Rv-3615c in the intradermal test and the interferon-γ assay for diagnosis of bovine tuberculosis. Preventive Veterinary Medicine, 105(1), 149–154. https://doi.org/10.1016/j.prevetmed.2012.02.007

Casal, C., Infantes, J. A., Risalde, M. A., Díez-Guerrier, A., Domínguez, M., Moreno, I., … Bezos, J. (2017). Antibody detection tests improve the sensitivity of tuberculosis diagnosis in cattle. Research in Veterinary Science, 112, 214–221. https://doi.org/10.1016/j.rvsc.2017.05.012

Casal, Díez-Guerrier, A., Álvarez, J., Rodriguez-Campos, S., Mateos, A., Linscott, R., … Aranaz, A. (2014). Strategic use of serology for the diagnosis of bovine tuberculosis after intradermal skin testing. Veterinary Microbiology, 170(3–4), 342–351. https://doi.org/10.1016/j.vetmic.2014.02.036

Casas Olascoaga, R. (2013). Antecedentes de la Tuberculosis Bovina en Uruguay, Periodo marzo 1888 – enero 1998. Servicio Medico Veterinario Del Uruguay, 49, 14–30.

Cassidy, J. P., Bryson, D. G., Pollock, J. M., Evans, R. T., Forster, F., & Neill, S. D. (1998). Early lesion formation in cattle experimentally infected with Mycobacterium bovis. Journal of Comparative Pathology, 119(1), 27–44.

Cho, Y. S., Jung, S. C., Kim, J. M., & Yoo, H. S. (2007). Enzyme-Linked Immunosorbent Assay of Bovine Tuberculosis by Crude Mycobacterial Protein 70. Journal of Immunoassay and Immunochemistry, 28(4), 409–418. https://doi.org/10.1080/15321810701603781

Ciaravino, G., García-Saenz, A., Cabras, S., Allepuz, A., Casal, J., García-Bocanegra, I., … Napp, S. (2018). Assessing the variability in transmission of bovine tuberculosis within Spanish cattle herds. Epidemics, 23, 110–120. https://doi.org/10.1016/j.epidem.2018.01.003

Ciaravino, Giovanna, Ibarra, P., Casal, E., Lopez, S., Espluga, J., Casal, J., … Allepuz, A. (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. https://doi.org/10.3389/fvets.2017.00202

Coad, M., Clifford, D., Rhodes, S. G., Hewinson, R. G., Vordermeier, H. M., & Whelan, A. O. (2010). Repeat tuberculin skin testing leads to desensitisation in naturally infected tuberculous cattle which is associated with elevated interleukin-10 and decreased interleukin-1 beta responses. Veterinary Research, 41(2), 14. https://doi.org/10.1051/vetres/2009062

Collins, J. D. (2006). Tuberculosis in cattle: Strategic planning for the future. 4th International Conference on Mycobacterium Bovis, 112(2), 369–381. https://doi.org/10.1016/j.vetmic.2005.11.041

Conlan, A. J. K., McKinley, T. J., Karolemeas, K., Pollock, E. B., Goodchild, A. V., Mitchell, A. P., … Wood, J. L. N. (2012). Estimating the Hidden Burden of Bovine Tuberculosis in Great Britain. PLOS Computational Biology, 8(10), e1002730. https://doi.org/10.1371/journal.pcbi.1002730

Cosivi, O., Grange, J. M., Daborn, C. J., Raviglione, M. C., Fujikura, T., Cousins, D., … Meslin, F. X. (1998). Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerg Infect Dis, 4. https://doi.org/10.3201/eid0401.980108

de Garine-Wichatitsky, M., Caron, A., Gomo, C., Foggin, C., Dutlow, K., Pfukenyi, D., … Michel, A. (2010). Bovine tuberculosis in buffaloes, Southern Africa. Emerging Infectious Diseases, 16(5), 884–885. https://doi.org/10.3201/eid1605.090710

de Jong, M. C. M. (1995). Mathematical modelling in veterinary epidemiology: why model building is important. Veterinary Epidemiology and Economics, 25(2), 183–193. https://doi.org/10.1016/0167-5877(95)00538-2

de Kantor, I. N., & Ritacco, V. (1994). Bovine tuberculosis in Latin America and the Caribbean: current status, control and eridication programs. Veterinary Microbiology, 40(1), 5–14. https://doi.org/10.1016/0378-1135(94)90042-6

de la Cruz, M. L., Branscum, A. J., Nacar, J., Pages, E., Pozo, P., Perez, A., … Alvarez, J. (2018). Evaluation of the Performance of the IDvet IFN-Gamma Test for Diagnosis of Bovine Tuberculosis in Spain. Frontiers in Veterinary Science, 5, 229–229. https://doi.org/10.3389/fvets.2018.00229

de la Rua-Domenech, R., Goodchild, A. T., Vordermeier, H. M., Hewinson, R. G., Christiansen, K. H., & Clifton-Hadley, R. S. (2006). 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. https://doi.org/10.1016/j.rvsc.2005.11.005

DIEA. (2018). Anuario Estadístico. Retrieved from https://descargas.mgap.gub.uy/DIEA/Anuarios/Anuario2018/Anuario_2018.pdf

Doherty, M. L., Bassett, H. F., Quinn, P. J., Davis, W. C., Kelly, A. P., & Monaghan, M. L. (1996). A sequential study of the bovine tuberculin reaction. Immunology, 87(1), 9–14. Retrieved from PMC. (PMC1383961)

Dohoo, I. R., Martin, S. W., & Stryhn, H. (2009). Veterinary epidemiologic research. Charlotte, P.E.I.: VER, Inc. /z-wcorg/.

Domingo, M., Vidal, E., & Marco, A. (2014). Pathology of bovine tuberculosis. Bovine Tuberculosis, 97, S20–S29. https://doi.org/10.1016/j.rvsc.2014.03.017

Drewe, J. A., Tomlinson, A. J., Walker, N. J., & Delahay, R. J. (2010). Diagnostic Accuracy and Optimal Use of Three Tests for Tuberculosis in Live Badgers. PLOS ONE, 5(6), e11196. https://doi.org/10.1371/journal.pone.0011196

Dunn, J. R., Kaneene, J. B., Grooms, D. L., Bolin, S. R., Bolin, C. A., & Bruning-Fann, C. S. (2005). Effects of positive results for Mycobacterium avium subsp paratuberculosis as determined by microbial culture of feces or antibody ELISA on results of caudal fold tuberculin test and interferon-γ assay for tuberculosis in cattle. Journal of the American Veterinary Medical Association, 226(3), 429–435. https://doi.org/10.2460/javma.2005.226.429

Eda, S., Bannantine, J. P., Waters, W. R., Mori, Y., Whitlock, R. H., Scott, M. C., & Speer, C. A. (2006). A Highly Sensitive and Subspecies-Specific Surface Antigen Enzyme- Linked Immunosorbent Assay for Diagnosis of Johne's Disease. Clinical and Vaccine Immunology, 13(8), 837. https://doi.org/10.1128/CVI.00148-06

EFSA (European Food Safety Authority). (2012). Scientific Opinion on the use of a gamma interferon test for the diagnosis of bovine tuberculosis.

EFSA Panel on Animal Health and Welfare (AHAW), More, S., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., … Bicout, D. (2017). Ad hoc method for the assessment on listing and categorisation of animal diseases within the framework of the Animal Health Law. EFSA Journal, 15(7), e04783–n/a. https://doi.org/10.2903/j.efsa.2017.4783

Enøe, C., Georgiadis, M. P., & Johnson, W. O. (2000). Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. Preventive Veterinary Medicine, 45(1), 61–81. https://doi.org/10.1016/S0167-5877(00)00117-3

Farnham, M. W., Norby, B., Goldsmith, T. J., & Wells, S. J. (2012a). Meta-analysis of field studies on bovine tuberculosis skin tests in United States cattle herds. Preventive Veterinary Medicine, 103(2–3), 234–242. https://doi.org/10.1016/j.prevetmed.2011.08.009

Farnham, M. W., Norby, B., Goldsmith, T. J., & Wells, S. J. (2012b). Meta-analysis of field studies on bovine tuberculosis skin tests in United States cattle herds. Preventive Veterinary Medicine, 103(2), 234–242. https://doi.org/10.1016/j.prevetmed.2011.08.009

Fitzgerald, S. D., & Kaneene, J. B. (2013). Wildlife Reservoirs of Bovine Tuberculosis Worldwide: Hosts, Pathology, Surveillance, and Control. Veterinary Pathology Online, 50(3), 488–499. https://doi.org/10.1177/0300985812467472

Flores-Villalva, S., Suárez-Güemes, F., Espitia, C., Whelan, A. O., Vordermeier, M., & Gutiérrez-Pabello, J. A. (2012). Specificity of the Tuberculin Skin Test Is Modified by Use of a Protein Cocktail Containing ESAT-6 and CFP-10 in Cattle Naturally Infected with Mycobacterium bovis. Clinical and Vaccine Immunology, 19(5), 797. https://doi.org/10.1128/CVI.05668-11

Gardner, I.A. (2002). The utility of Bayes' theorem and Bayesian inference in veterinary clinical practice and research. Australian Veterinary Journal, 80(12), 758–761. https://doi.org/10.1111/j.1751-0813.2002.tb11347.x

Gardner, Ian A, Stryhn, H., Lind, P., & Collins, M. T. (2000). Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. Preventive Veterinary Medicine, 45(1), 107–122. https://doi.org/10.1016/S0167-5877(00)00119-7

Gelman, A, & Hill, J. (2007). Data analysis using regression and multilevel/hierarchical models (Cambridge). New York: Cambridge University Press.

Gelman, Andrew, & Rubin, D. B. (1992). Inference from Iterative Simulation Using Multiple Sequences. Statistical Science, 7(4), 457–472.

Georgiadis, M. P., Johnson, W. O., Gardner, I. A., & Singh, R. (2003). Correlation-adjusted estimation of sensitivity and specificity of two diagnostic tests. Journal of the Royal Statistical Society: Series C (Applied Statistics), 52(1), 63–76. https://doi.org/10.1111/1467-9876.00389

Gilardoni, L., Paolicchi, F., & Mundo, S. (2012). Bovine paratuberculosis: a review of the advantages and disadvantages of different diagnostic tests. Revista Argentina De Microbiologia, 44(3), 201–215.

Gillespie, D. T. (2001). Approximate accelerated stochastic simulation of chemically reacting systems. The Journal of Chemical Physics, 115(4), 1716–1733. https://doi.org/10.1063/1.1378322

Gilot, P., & Cocito, C. (1993). Comparative analysis of three sensitins used in cutaneous testing for tuberculosis and paratuberculosis in cattle. FEMS Microbiology Letters, 110(3), 307–311. https://doi.org/10.1111/j.1574-6968.1993.tb06340.x

Good, M., Bakker, D., Duignan, A., & Collins, D. M. (2018). The History of In Vivo Tuberculin Testing in Bovines: Tuberculosis, a "One Health" Issue. Frontiers in Veterinary Science, 5, 59–59. https://doi.org/10.3389/fvets.2018.00059

Gormley, A. M., Anderson, D. P., & Nugent, G. (2017). Cost-based optimization of the stopping threshold for local disease surveillance during progressive eradication of tuberculosis from New Zealand wildlife. Transboundary and Emerging Diseases, n/a-n/a. https://doi.org/10.1111/tbed.12647

Gormley, E., Corner, L. A. L., Costello, E., & Rodriguez-Campos, S. (2014). Bacteriological diagnosis and molecular strain typing of Mycobacterium bovis and Mycobacterium caprae. Bovine Tuberculosis, 97, Supplement, S30–S43. https://doi.org/10.1016/j.rvsc.2014.04.010

Gormley, E., Doyle, M. B., Fitzsimons, T., McGill, K., & Collins, J. D. (2006). Diagnosis of Mycobacterium bovis infection in cattle by use of the gamma-interferon (Bovigam®) assay. 4th International Conference on Mycobacterium Bovis, 112(2), 171–179. https://doi.org/10.1016/j.vetmic.2005.11.029

Gormley, E., Doyle, M. B., McGill, K., Costello, E., Good, M., & Collins, J. D. (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), 413–420. https://doi.org/10.1016/j.vetimm.2004.08.002

Gortazar, C., Vicente, J., Boadella, M., Ballesteros, C., Galindo, R. C., Garrido, J., … de la Fuente, J. (2011). Progress in the control of bovine tuberculosis in Spanish wildlife. Veterinary Microbiology, 151(1–2), 170–178. https://doi.org/10.1016/j.vetmic.2011.02.041

Green, L. R., Jones, C. C., Sherwood, A. L., Garkavi, I. V., Cangelosi, G. A., Thacker, T. C., … Rathe, C. V. (2009). Single-Antigen Serological Testing for Bovine Tuberculosis. Clinical and Vaccine Immunology, 16(9), 1309. https://doi.org/10.1128/CVI.00028-09

Halasa, T., & Dürr, S. (2017). Editorial: Modeling Disease Spread and Control. Frontiers in Veterinary Science, 4, 199. https://doi.org/10.3389/fvets.2017.00199

Hope, J. C., Thom, M. L., Villarreal-Ramos, B., Vordermeier, H. M., Hewinson, R. G., & Howard, C. J. (2005). Exposure to Mycobacterium avium induces low-level protection from Mycobacterium bovis infection but compromises diagnosis of disease in cattle. Clinical & Experimental Immunology, 141(3), 432–439. https://doi.org/10.1111/j.1365-2249.2005.02882.x

Humblet, M.-F., Boschiroli, M., & Saegerman, C. (2009). Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Vet. Res., 40(5), 50.

Jenkins, A. O., Cadmus, S. I. B., Venter, E. H., Pourcel, C., Hauk, Y., Vergnaud, G., & Godfroid, J. (2011). Molecular epidemiology of human and animal tuberculosis in Ibadan, Southwestern Nigeria. Veterinary Microbiology, 151(1–2), 139–147. https://doi.org/10.1016/j.vetmic.2011.02.037

Kao R. R., Roberts M. G., & 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), 1069–1076. https://doi.org/10.1098/rspb.1997.0148

Kao, S.-Y. Z., VanderWaal, K., Enns, E. A., Craft, M. E., Alvarez, J., Picasso, C., & Wells, S. J. (2018). Modeling cost-effectiveness of risk-based bovine tuberculosis surveillance in Minnesota. Preventive Veterinary Medicine, 159, 1–11. https://doi.org/10.1016/j.prevetmed.2018.08.011

Keck, N., Dutruel, H., Smyej, F., Nodet, M., & Boschiroli, M. L. (2010). Tuberculosis due to Mycobacterium bovis in a Camargue horse. Veterinary Record, 166(16), 499. https://doi.org/10.1136/vr.b4785

Keeling, M. J., & Rohani, P. (2008). Modelling Infectious Diseases in Human and Animals. Princeton University Press.

Kennedy, A.E., Byrne, N., O'Mahony, J., & Sayers, R. G. (2017). Investigations and implications of associations between mycobacterial purified protein derivative hypersensitivity and MAP-antibody ELISA in Irish dairy cows. Research in Veterinary Science, 115, 13–16. https://doi.org/10.1016/j.rvsc.2017.01.018

Kennedy, Aideen E., Da Silva, A. T., Byrne, N., Govender, R., MacSharry, J., O'Mahony, J., & Sayers, R. G. (2014). The single intradermal cervical comparative test interferes with Johne's disease ELISA diagnostics.

Kennedy, Aideen E, O'Mahony, J., Byrne, N., MacSharry, J., & Sayers, R. G. (2017). Is TB Testing Associated With Increased Blood Interferon-Gamma Levels? Frontiers in Veterinary Science, 4, 176. https://doi.org/10.3389/fvets.2017.00176

Kindt, T. J., Osborne, B. A., & Goldsby, R. A. (2007). Immunology. New York; Basingstoke: W.H. Freeman ; Palgrave [distributor]. /z-wcorg/.

Kostoulas, P., Browne, W. J., Nielsen, S. S., & Leontides, L. (2013). Bayesian mixture models for partially verified data: Age- and stage-specific discriminatory power of an antibody ELISA for paratuberculosis. Preventive Veterinary Medicine, 111(3), 200–205. https://doi.org/10.1016/j.prevetmed.2013.05.006

Kostoulas, P., Nielsen, S. S., Branscum, A. J., Johnson, W. O., Dendukuri, N., Dhand, N. K., … Gardner, I. A. (2017). STARD-BLCM: Standards for the Reporting of Diagnostic accuracy studies that use Bayesian Latent Class Models. Preventive Veterinary Medicine, 138, 37–47. https://doi.org/10.1016/j.prevetmed.2017.01.006

Kruskal, W. H., & Wallis, W. A. (1952). Use of Ranks in One-Criterion Variance Analysis. Journal of the American Statistical Association, 47(260), 583–621. https://doi.org/10.2307/2280779

Lilenbaum, W., Marassi, C. D., Varges, R., Medeiros, L., Oelemann, W. M. R., & Fonseca, L. S. (2009). Occurrence of false-positive results in three Paratuberculosis - ELISAs performed in a tuberculous herd. Veterinary Research Communications, 33(7), 693–699. https://doi.org/10.1007/s11259-009-9218-3

Lilenbaum, Walter, Ferreira, R., Marassi, C. D., Ristow, P., Oelemann, W. M. R., & Fonseca, L. de S. (2007). Interference of tuberculosis on the performance of ELISAs used in the diagnosis of paratuberculosis in cattle. Brazilian Journal of Microbiology, 38, 472–477.

Lisle, G. de, Yates, G., Caley, P., & Corboy, R. (2005). Surveillance of wildlife for Mycobacterium bovis infection using culture of pooled tissue samples from ferrets (Mustela furo). New Zealand Veterinary Journal, 53(1), 14–18. https://doi.org/10.1080/00480169.2005.36463

Lunn, D., Spiegelhalter, D., Thomas, A., & Best, N. (2009). The BUGS project: Evolution, critique and future directions. Statistics in Medicine, 28(25), 3049–3067. https://doi.org/10.1002/sim.3680

Lyashchenko, K., Colangeli, R., Houde, M., Al Jahdali, H., Menzies, D., & Gennaro, M. L. (1998). Heterogeneous Antibody Responses in Tuberculosis. Infection and Immunity, 66(8), 3936.

Lyashchenko, Konstantin P., Pollock, J. M., Colangeli, R., & Gennaro, M. L. (1998). Diversity of Antigen Recognition by Serum Antibodies in Experimental Bovine Tuberculosis. Infection and Immunity, 66(11), 5344.

Lyashchenko, K.P., Greenwald, R., Esfandiari, J., Chambers, M. A., Vicente, J., Gortazar, C., … Waters, W. R. (2008). Animal-side serologic assay for rapid detection of Mycobacterium bovis infection in multiple species of free-ranging wildlife. Veterinary Microbiology, 132(3), 283–292. https://doi.org/10.1016/j.vetmic.2008.05.029

Machado, G., Kanankege, K., Schumann, V., Wells, S., Perez, A., & Alvarez, J. (2018). Identifying individual animal factors associated with Mycobacterium avium subsp. paratuberculosis (MAP) milk ELISA positivity in dairy cattle in the Midwest region of the United States. BMC Veterinary Research, 14, 28. https://doi.org/10.1186/s12917-018-1354-y

Mckenna, S. L. B., Sockett, D. C., Keefe, G. P., Mcclure, J., Vanleeuwen, J. A., & Barkema, H. W. (2005). Comparison of Two Enzyme-Linked Immunosorbent Assays for Diagnosis of Mycobacterium Avium Subsp. Paratuberculosis. Journal of Veterinary Diagnostic Investigation, 17(5), 463–466. https://doi.org/10.1177/104063870501700510

McMurray, J. (1941). The Incidence of Bovine Tuberculosis in Humans in Northern Ireland. The Ulster Medical Journal, 10(2), 132–114.12. Retrieved from PubMed. (20476276)

Mcnair, J., Corbett, D. M., Girvin, R. M., Mackie, D. P., & Pollock, J. M. (2001). Characterization of the Early Antibody Response in Bovine Tuberculosis: MPB83 is an Early Target with Diagnostic Potential. Scandinavian Journal of Immunology, 53(4), 365–371. https://doi.org/10.1046/j.1365-3083.2001.00874.x

Menzies, F. D., & Neill, S. D. (2000). Cattle-to-cattle transmission of bovine tuberculosis. Veterinary Journal (London, England : 1997), 160(2), 92–106. https://doi.org/10.1053/tvjl.2000.0482

Menzies, Fraser D., Abernethy, D. A., Stringer, L. A., Honhold, N., & Gordon, A. W. (2012). A matched cohort study investigating the risk of Mycobacterium bovis infection in the progeny of infected cows. Veterinary Journal (London, England : 1997), 194(3), 299–302. https://doi.org/10.1016/j.tvjl.2012.05.005

MGAP, M. de G. A. y P. (1989). Legislacion Sanitaria Animal. Retrieved from http://www2.mgap.gub.uy/portal/page.aspx?2,dgsg,dgsg-legislacion-sanitaria

Miller, R. S., & Sweeney, S. J. (2013). Mycobacterium bovis (bovine tuberculosis) infection in North American wildlife: current status and opportunities for mitigation of risks of further infection in wildlife populations. Epidemiology and Infection, 141(7), 1357–1370. https://doi.org/10.1017/S0950268813000976

Ministerio de Agricultura y Pesca AyMA. (2017). Programa de Erradicación de Tuberculosis Bovina presentado por España para el año 2017. Retrieved from http://www.mapama.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/pnetb_2017_3_tcm7-443753.pdf.

Monaghan, M. L., Doherty, M. L., Collins, J. D., Kazda, J. F., & Quinn, P. J. (1994). The tuberculin test. Veterinary Microbiology, 40(1), 111–124. https://doi.org/10.1016/0378-1135(94)90050-7

More, S. J., Radunz, B., & Glanville, R. J. (2015). Lessons learned during the successful eradication of bovine tuberculosis from Australia. Veterinary Record, 177(9), 224–232.

Morris, Roger S. (2015). Diseases, dilemmas, decisions—Converting epidemiological dilemmas into successful disease control decisions. Preventive Veterinary Medicine, 122(1), 242–252. https://doi.org/10.1016/j.prevetmed.2015.05.003

Morris, R.S., Pfeiffer, D. U., & Jackson, R. (1994). The epidemiology of Mycobacterium bovis infections. Veterinary Microbiology, 40(1), 153–177. https://doi.org/10.1016/0378-1135(94)90053-1

Mosavari, N., Geravand, M. M., Tadayon, K., & Keshavarz, R. (2016). Mycobacterial coinfection and persisting bovine tuberculosis—Has the time arrived for a policy review? Supplement: The 2nd Asian-African Congress of International Journal of Mycobacteriology, Iran, 5, S82–S83. https://doi.org/10.1016/j.ijmyco.2016.09.016

Muller, B., Durr, S., Alonso, S., Hattendorf, J., Laisse, C. J. M., Parsons, S. D. C., … Zinsstag, J. (2013). Zoonotic Mycobacterium bovis-induced tuberculosis in humans. Emerging Infectious Diseases, 19(6), 899+. Retrieved from Expanded Academic ASAP.

Müller, B., Dürr, S., Alonso, S., Hattendorf, J., Laisse, C. J., Parsons, S. D., … Zinsstag, J. (2013). Zoonotic Mycobacterium bovis–induced Tuberculosis in Humans. Emerging Infectious Diseases, 19(6), 899–908. https://doi.org/10.3201/eid1906.120543

Muñoz Mendoza, M., Juan, L. de, Menéndez, S., Ocampo, A., Mourelo, J., Sáez, J. L., … Balseiro, A. (2012). Tuberculosis due to Mycobacterium bovis and Mycobacterium caprae in sheep. The Veterinary Journal, 191(2), 267–269. https://doi.org/10.1016/j.tvjl.2011.05.006

Neill, S. D., Bryson, D. G., & Pollock, J. M. (2001). Pathogenesis of tuberculosis in cattle. Tuberculosis, 81(1), 79–86. https://doi.org/10.1054/tube.2000.0279

Neill, S. D., Hanna, J., O'Brien, J. J., & McCracken, R. M. (1988). Excretion of Mycobacterium bovis by experimentally infected cattle. The Veterinary Record, 123(13), 340–343.

Neill, S. D., O'Brien, J. J., & Hanna, J. (1991). A mathematical model for Mycobacterium bovis excretion from tuberculous cattle. Veterinary Microbiology, 28(1), 103–109. https://doi.org/10.1016/0378-1135(91)90102-L

Neill, S. D., Pollock, J. M., Bryson, D. B., & Hanna, J. (1994). Pathogenesis of Mycobacterium bovis infection in cattle. Veterinary Microbiology, 40(1–2), 41–52.

Nielsen, Søren Saxmose, & Toft, N. (2009). A review of prevalences of paratuberculosis in farmed animals in Europe. Preventive Veterinary Medicine, 88(1), 1–14. https://doi.org/10.1016/j.prevetmed.2008.07.003

Nielsen, Søren Saxmose, Toft, N., & Okura, H. (2013). Dynamics of Specific Anti-Mycobacterium avium Subsp. paratuberculosis Antibody Response through Age. PLoS ONE, 8(4), e63009. https://doi.org/10.1371/journal.pone.0063009

Nielsen, S.S., & Toft, N. (2008). Ante mortem diagnosis of paratuberculosis: A review of accuracies of ELISA, interferon-Oi assay and faecal culture techniques. Veterinary Microbiology, 129(3–4), 217–235. https://doi.org/10.1016/j.vetmic.2007.12.011

Norby, B., Bartlett, P. C., Fitzgerald, S. D., Granger, L. M., Bruning-Fann, C. S., Whipple, D. L., & Payeur, J. B. (2004). The Sensitivity of Gross Necropsy, Caudal Fold and Comparative Cervical Tests for the Diagnosis of Bovine Tuberculosis. Journal of Veterinary Diagnostic Investigation, 16(2), 126–131. https://doi.org/10.1177/104063870401600206

Nugent, G., Buddle, B. M., & Knowles, G. (2015). Epidemiology and control of Mycobacterium bovis infection in brushtail possums (Trichosurus vulpecula), the primary wildlife host of bovine tuberculosis in New Zealand. New Zealand Veterinary Journal, 63 Suppl 1(sup1), 28–41. https://doi.org/10.1080/00480169.2014.963791

Nuñez-Garcia, J., Downs, S. H., Parry, J. E., Abernethy, D. A., Broughan, J. M., Cameron, A. R., … Greiner, M. (2018). Meta-analyses of the sensitivity and specificity of ante-mortem and post-mortem diagnostic tests for bovine

tuberculosis in the UK and Ireland. Preventive Veterinary Medicine, 153, 94–107. https://doi.org/10.1016/j.prevetmed.2017.02.017

OIE. (2009). Bovine tuberculosis. 2009. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2014. World Organisation for Animal Health. Retrieved from http://www. oie.int/international-standard-setting/terrestrial-manual/access-online/

OIE. (n.d.). Terrestrial Animal Heath Code. Retrieved from http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_bovine_tuberculosis.htm

Olea-Popelka, F., Muwonge, A., Perera, A., Dean, A. S., Mumford, E., Erlacher-Vindel, E., … Fujiwara, P. I. (2017). Zoonotic tuberculosis in human beings caused by Mycobacterium bovis—a call for action. The Lancet Infectious Diseases, 17(1), e21–e25. https://doi.org/10.1016/S1473-3099(16)30139-6

Ott, S. L., Wells, S. J., & Wagner, B. A. (1999). Herd-level economic losses associated with Johne's disease on US dairy operations. Preventive Veterinary Medicine, 40(3–4), 179–192. https://doi.org/10.1016/S0167-5877(99)00037-9

Over, K., Crandall, P. G., O'Bryan, C. A., & Ricke, S. C. (2011). Current perspectives on Mycobacterium avium subsp. paratuberculosis, Johne's disease, and Crohn's disease: a Review. Critical Reviews in Microbiology, 37(2), 141–156. https://doi.org/10.3109/1040841X.2010.532480

Palmer, M V, Waters, W. R., Thacker, T. C., Greenwald, R., Esfandiari, J., & Lyashchenko, K. P. (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), 387–394. https://doi.org/10.1128/CVI.13.3.387-394.2006

Palmer, Mitchell V, & Waters, W. R. (2011). Bovine tuberculosis and the establishment of an eradication program in the United States: role of veterinarians. Veterinary Medicine International, 2011, 816345–816345. https://doi.org/10.4061/2011/816345

Pearce, B. H., Fosgate, G. T., Ward, M. P., Roussel, A. J., Norby, B., Tavornpanich, S., & Ellis, D. (2008). Comparison of three methods of surveillance with application to the detection of Johne's disease seropositivity in Texas cattle.

Preventive Veterinary Medicine, 86(1), 1–7. https://doi.org/10.1016/j.prevetmed.2008.03.002

Perez, A. M., Ward, M. P., & Ritacco, V. (2002). Simulation-model evaluation of bovine tuberculosis-eradication strategies in Argentine dairy herds. Preventive Veterinary Medicine, 54(4), 351–360. https://doi.org/10.1016/S0167-5877(02)00044-2

Pfeiffer, D. U. (2013). Epidemiology Caught in the Causal Web of Bovine Tuberculosis. Transboundary and Emerging Diseases, 60, 104–110. https://doi.org/10.1111/tbed.12105

Phillips, C. J. C., Foster, C. R. W., Morris, P. A., & Teverson, R. (2003). The transmission of Mycobacterium bovis infection to cattle. Research in Veterinary Science, 74(1), 1–15.

Picasso, C., Alvarez, J., VanderWaal, K. L., Fernandez, F., Gil, A., Wells, S. J., & Perez, A. (2017). Epidemiological investigation of bovine tuberculosis outbreaks in Uruguay (2011–2013). Preventive Veterinary Medicine, 138, 156–161. https://doi.org/10.1016/j.prevetmed.2017.01.010

Picasso-Risso, C., Gil, A., Nunez, A., Suanes, A., Macchi, V., Salaberry, X., … Perez, A. (2019). Diagnostic interaction between bovine tuberculosis (bTB) and Johne's disease in bTB highly prevalent dairy farms of Uruguay. Veterinary and Animal Science, 100052. https://doi.org/10.1016/j.vas.2019.100052

Pollock, J. M., McNair, J., Welsh, M. D., Girvin, R. M., Kennedy, H. E., Mackie, D. P., & Neill, S. D. (2001). Immune responses in bovine tuberculosis. Tuberculosis, 81(1), 103–107. https://doi.org/10.1054/tube.2000.0258

Pollock, J. M., Welsh, M. D., & McNair, J. (2005). Immune responses in bovine tuberculosis: Towards new strategies for the diagnosis and control of disease. 7th International Veterinary Immunology Symposium, 108(1), 37–43. https://doi.org/10.1016/j.vetimm.2005.08.012

Praud, A., BOSCHIROLI, M. L., MEYER, L., GARIN-BASTUJI, B., & DUFOUR, B. (2015). Assessment of the sensitivity of the gamma-interferon test and the single intradermal comparative cervical test for the diagnosis of bovine tuberculosis under field conditions. Epidemiology and Infection, 143(1), 157–166. https://doi.org/10.1017/S0950268814000338

Radunz, B., & Lepper. (1985). Suppression of skin reactivity to bovine tuberculin in repeat tests. Australian Veterinary Journal, 62(6), 191–194. https://doi.org/10.1111/j.1751-0813.1985.tb07294.x

Rangel, S. J., Paré, J., Doré, E., Arango, J. C., Côté, G., Buczinski, S., … Fecteau, G. (2015). A systematic review of risk factors associated with the introduction of Mycobacterium avium spp. paratuberculosis (MAP) into dairy herds. The Canadian Veterinary Journal, 56(2), 169–177. Retrieved from PMC. (PMC4298270)

Riella, A., & RamÃ\-rez, J. (2012). La calidad del empleo en la ganaderÃ\-a uruguaya: Un estudio de caso. Agrociencia Uruguay, 16, 186–197.

Ritacco, V., Lopez, B., De Kantor, I. N., Barrera, L., Errico, F., & Nader, A. (1991). Reciprocal cellular and humoral immune responses in bovine tuberculosis. Research in Veterinary Science, 50(3), 365–367. https://doi.org/10.1016/0034-5288(91)90143-C

Robinson, Orme, I. M., & Cooper, A. M. (2015). The onset of adaptive immunity in the mouse model of tuberculosis and the factors that compromise its expression. Immunological Reviews, 264(1), 46–59. https://doi.org/10.1111/imr.12259

Robinson, P. A. (2015). A history of bovine tuberculosis eradication policy in Northern Ireland. Epidemiology & Infection, 143(15), 3182–3195.

Rodriguez, E., Sanchez, L. P., Perez, S., Herrera, L., Jimenez, M. S., Samper, S., & Iglesias, M. J. (2009). Human tuberculosis due to Mycobacterium bovis and M. caprae in Spain, 2004-2007. The International Journal of Tuberculosis and Lung Disease : The Official Journal of the International Union against Tuberculosis and Lung Disease, 13(12), 1536–1541.

Rogan, W., & Gladen, B. (1978). ESTIMATING PREVALENCE FROM THE RESULTS OF A SCREENING TEST. American Journal of Epidemiology, 107(1), 71–76. https://doi.org/10.1093/oxfordjournals.aje.a112510

Rossi, G., Aubry, P., Dubé, C., & Smith, R. L. (2019). The spread of bovine tuberculosis in Canadian shared pastures: Data, model, and simulations. Transboundary and Emerging Diseases, 66(1), 562–577. https://doi.org/10.1111/tbed.13066

Rossi, G., Aubry, P., Dube, C., & Smith, R. L. (2019). The spread of bovine tuberculosis in Canadian shared pastures: Data, model, and simulations. Transboundary and Emerging Diseases, 66(1), 562. https://doi.org/10.1111/tbed.13066

Rothel, J., JONES, S., CORNER, L., COX, J., & WOOD, P. (1992). The gamma-interferon assay for diagnosis of bovine tuberculosis in cattle: conditions affecting the production of gamma-interferon in whole blood culture. Australian Veterinary Journal, 69(1), 1–4. https://doi.org/10.1111/j.1751-0813.1992.tb09848.x

Roupie, V., Alonso-Velasco, E., Van Der Heyden, S., Holbert, S., Duytschaever, L., Berthon, P., … Fretin, D. (2018). Evaluation of mycobacteria-specific gamma interferon and antibody responses before and after a single intradermal skin test in cattle naturally exposed to M. avium subsp. paratuberculosis and experimentally infected with M. bovis. Veterinary Immunology and Immunopathology, 196, 35–47. https://doi.org/10.1016/j.vetimm.2017.12.007

Roussel, A. J., Fosgate, G. T., Manning, E. J. B., & Collins, M. T. (2007). Association of fecal shedding of mycobacteria with high ELISA-determined seroprevalence for paratuberculosis in beef herds. Journal of the American Veterinary Medical Association, 230(6), 890–895. https://doi.org/10.2460/javma.230.6.890

Rua-Domenech, R. (2006). Human Mycobacterium bovis infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. Tuberculosis (Edinb), 86. https://doi.org/10.1016/j.tube.2005.05.002

Rutjes, A. W. S., Reitsma, J. B., Vandenbroucke, J. P., Glas, A. S., & Bossuyt, P. M. M. (2005). Case–Control and Two-Gate Designs in Diagnostic Accuracy Studies. Clinical Chemistry, 51(8), 1335. https://doi.org/10.1373/clinchem.2005.048595

Sarradell, J. E., Alvarez, J., Biscia, M., Zumarraga, M., Wunschmann, A., Armien, A. G., & Perez, A. M. (2015). Mycobacterium bovis infection in a horse with granulomatous enterocolitis. Journal of Veterinary Diagnostic Investigation : Official Publication of the American Association of Veterinary Laboratory

Diagnosticians, Inc, 27(2), 203–205. https://doi.org/10.1177/1040638715571359

Schiller, I., Oesch, B., Vordermeier, H. M., Palmer, M. V., Harris, B. N., Orloski, K. A., … Waters, W. R. (2010). Bovine Tuberculosis: A Review of Current and Emerging Diagnostic Techniques in View of their Relevance for Disease Control and Eradication. Transboundary and Emerging Diseases, 57(4), 205–220. https://doi.org/10.1111/j.1865-1682.2010.01148.x

Schiller, Irene, Vordermeier, H. M., Waters, W. R., Whelan, A. O., Coad, M., Gormley, E., … Oesch, B. (2010). Bovine tuberculosis: Effect of the tuberculin skin test on in vitro interferon gamma responses. Veterinary Immunology and Immunopathology, 136(1–2), 1–11. https://doi.org/10.1016/j.vetimm.2010.02.007

Schmiedel, A. (1968). Development and present state of bovine tuberculosis in man. Bulletin of the International Union against Tuberculosis, 40, 5–32.

Scott, M. C., Bannantine, J. P., Kaneko, Y., Branscum, A. J., Whitlock, R. H., Mori, Y., … Eda, S. (2010). Absorbed EVELISA: A Diagnostic Test with Improved Specificity for Johne's Disease in Cattle. Foodborne Pathogens and Disease, 7(11), 1291–1296.

Seva, J., Sanes, J. M., Ramis, G., Mas, A., Quereda, J. J., Villarreal-Ramos, B., … Pallares, F. J. (2014). Evaluation of the single cervical skin test and interferon gamma responses to detect Mycobacterium bovis infected cattle in a herd co-infected with Mycobacterium avium subsp. paratuberculosis. Veterinary Microbiology, 171(1), 139–146. https://doi.org/10.1016/j.vetmic.2014.03.035

Shury, T. K., Nishi, J. S., Elkin, B. T., & Wobeser, G. A. (2015). TUBERCULOSIS AND BRUCELLOSIS IN WOOD BISON (BISON BISON ATHABASCAE) IN NORTHERN CANADA: A RENEWED NEED TO DEVELOP OPTIONS FOR FUTURE MANAGEMENT. Journal of Wildlife Diseases, 51(3), 543–554. https://doi.org/10.7589/2014-06-167

Singh, A. V., Chauhan, D., & singh, A. (2016). Mixed infection of bovine tuberculosis and paratuberculosis in domestic livestock species of North India. International Journal of Infectious Diseases, 45, 475. https://doi.org/10.1016/j.ijid.2016.02.1002

Smith, R. L., Schukken, Y. H., Lu, Z., Mitchell, R. M., & Grohn, Y. T. (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), 411–423. https://doi.org/10.2460/javma.243.3.411

Smith, R. L., Tauer, L. W., Schukken, Y. H., Lu, Z., & Grohn, Y. T. (2013). Minimization of bovine tuberculosis control costs in US dairy herds. Preventive Veterinary Medicine, 112(3–4), 266–275. https://doi.org/10.1016/j.prevetmed.2013.07.014

Snider, D. E. (1982). The Tuberculin Skin Test. American Review of Respiratory Disease, 125(3P2), 108–118. https://doi.org/10.1164/arrd.1982.125.3P2.108

Speer, C. A., Scott, M. C., Bannantine, J. P., Waters, W. R., Mori, Y., Whitlock, R. H., & Eda, S. (2006). A Novel Enzyme-Linked Immunosorbent Assay for Diagnosis of Mycobacterium avium subsp. paratuberculosis Infections (Johne's Disease) in Cattle. Clinical and Vaccine Immunology, 13(5), 535. https://doi.org/10.1128/CVI.13.5.535-540.2006

Spiegelhalter, D. J., Best, N. G., Carlin, B. P., & van der Linde, A. (2002). Bayesian Measures of Model Complexity and Fit. Journal of the Royal Statistical Society. Series B (Statistical Methodology), 64(4), 583–639.

Spiegelhalter, D., Thomas, A., Best, N. G., & Gilks, W. (1996). BUGS: Bayesian inference using Gibbs sampling, version 0.50. MRC Biostatistics Unit, Cambridge.

Stott, A. W., Jones, G. M., Humphry, R. W., & Gunn, G. J. (2005). Financial incentive to control paratuberculosis (Johne's disease) on dairy farms in the United Kingdom. Veterinary Record, 156(26), 825–831. https://doi.org/10.1136/vr.156.26.825

Sturtz, S., Ligges, U., & Gelman, A. (2005). A Package for Running WinBUGS from R. Journal of Statistical Software (Version 12 (3) 1-16).

Suanes, A., Nuñez, A., Piaggio, J., Picasso, C., Salaberry, X., Gil, A., … Fernandez, F. (2018, June 4). Johne's disease studies in Uruguayan dairy cattle between

1998 and 2015. Preliminary findings. Proceedings of the 14th International Colloquium on Paratuberculosis. Rivera Maya, Mexico.

Suess, E., Gardner, I., & Johnson, W. (2002). Hierarchical Bayesian model for prevalence inferences and determination of a country's status for an animal pathogen. Preventive Veterinary Medicine, 55(3), 155–171. https://doi.org/10.1016/s0167-5877(02)00092-2

Sweeney, R. W. (2011). Pathogenesis of Paratuberculosis. Johne's Disease, 27(3), 537–546. https://doi.org/10.1016/j.cvfa.2011.07.001

Thoen, C. O., Karlson, A. G., & Himes, E. M. (1981). Mycobacterial Infections in Animals. Clinical Infectious Diseases, 3(5), 960–972. https://doi.org/10.1093/clinids/3.5.960

Thom, M., Morgan, J. H., Hope, J. C., Villarreal-Ramos, B., Martin, M., & Howard, C. J. (2004). The effect of repeated tuberculin skin testing of cattle on immune responses and disease following experimental infection with Mycobacterium bovis. Veterinary Immunology and Immunopathology, 102(4), 399–412. https://doi.org/10.1016/j.vetimm.2004.08.005

Thomas, A., O'Hara, B., Ligges, U., & Sturtz, S. (2006). Making BUGS Open. R News, 6, 12–17.

Tschopp, R., Hattendorf, J., Roth, F., Choudhoury, A., Shaw, A., Aseffa, A., & Zinsstag, J. (2013). Cost Estimate of Bovine Tuberculosis to Ethiopia. In S. J. Mackenzie, M. Jeggo, P. Daszak, & A. J. Richt (Eds.), One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases: The Concept and Examples of a One Health Approach (pp. 249–268). Retrieved from http://dx.doi.org/10.1007/82_2012_245

Valerie-Beau Pucken, Knubben-Schweizer, G., Döpfer, D., Groll, A., Hafner-Marx, A., Hörmansdorfer, S., … Hartnack, S. (2017). Evaluating diagnostic tests for bovine tuberculosis in the southern part of Germany: A latent class analysis. PLoS One, 12(6). https://doi.org/10.1371/journal.pone.0179847

VanderWaal, K., Enns, E. A., Picasso, C., Alvarez, J., Perez, A., Fernandez, F., … Wells, S. (2017). Optimal surveillance strategies for bovine tuberculosis in a low-prevalence country. Scientific Reports, 7(1), 4140. https://doi.org/10.1038/s41598-017-04466-2

VanderWaal, K., Picasso, C., Enns, E. A., Craft, M. E., Alvarez, J., Fernandez, F., … Wells, S. (2016). Network analysis of cattle movements in Uruguay: Quantifying heterogeneity for risk-based disease surveillance and control. Preventive Veterinary Medicine, 123, 12–22. https://doi.org/10.1016/j.prevetmed.2015.12.003

Varges, R., Marassi, C. D., Oelemann, W., & Lilenbaum, W. (2009). Interference of intradermal tuberculin tests on the serodiagnosis of paratuberculosis in cattle. Research in Veterinary Science, 86(3), 371–372. https://doi.org/10.1016/j.rvsc.2008.08.006

Verteramo Chiu, L. J., Tauer, L. W., Smith, R. L., & Grohn, Y. T. (2019). Assessment of the bovine tuberculosis elimination protocol in the United States. Journal of Dairy Science, 102(3), 2384–2400. https://doi.org/10.3168/jds.2018-14990

Vordemeier, M., Goodchild, A. V., Whelan, A., Ewer, K., Clifton-Hadley, R. S., Williams, J., & Hewinson, R. G. (2006). The Bovigam assay as ancillary test to the tuberculin skin test. Government Veterinary Journal, 16, 72–80.

Vordermeier, H. M., Whelan, A., Cockle, P. J., Farrant, L., Palmer, N., & Hewinson, R. G. (2001). Use of Synthetic Peptides Derived from the Antigens ESAT-6 and CFP-10 for Differential Diagnosis of Bovine Tuberculosis in Cattle. Clinical and Diagnostic Laboratory Immunology, 8(3), 571–578. https://doi.org/10.1128/CDLI.8.3.571-578.2001

Vural, S. A., & Tunca, R. (2001). Generalized tuberculosis in a 45 day-old calf. DTW. Deutsche Tierarztliche Wochenschrift, 108(11), 468–470.

WAHIS_OIE. (2014). Animal health situation. http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation.

Waters, W R, Buddle, B. M., Vordermeier, H. M., Gormley, E., Palmer, M. V., Thacker, T. C., … Lawrence, J. C. (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), 1882–1888. https://doi.org/10.1128/CVI.05343-11

Waters, W. R., Palmer, M. V., Thacker, T. C., Bannantine, J. P., Vordermeier, H. M., Hewinson, R. G., … Lyashchenko, K. P. (2006). Early Antibody Responses to

Experimental Mycobacterium bovis Infection of Cattle. Clinical and Vaccine Immunology, 13(6), 648–654.

Waters, W Ray, Palmer, M. V., Stafne, M. R., Bass, K. E., Maggioli, M. F., Thacker, T. C., … Lyashchenko, K. P. (2015). Effects of Serial Skin Testing with Purified Protein Derivative on the Level and Quality of Antibodies to Complex and Defined Antigens in Mycobacterium bovis-Infected Cattle. Clinical and Vaccine Immunology : CVI, 22(6), 641–649. https://doi.org/10.1128/CVI.00119-15

Whelan, C., Shuralev, E., O'Keeffe, G., Hyland, P., Kwok, H. F., Snoddy, P., … Clarke, J. (2008). Multiplex immunoassay for serological diagnosis of Mycobacterium bovis infection in cattle. Clinical and Vaccine Immunology : CVI, 15(12), 1834–1838. https://doi.org/10.1128/CVI.00238-08

Whipple, D. L., Bolin, C. A., Davis, A. J., Jarnagin, J. L., Johnson, D. C., Nabors, R. S., … Wolf, M. M. (1995). Comparison of the sensitivity of the caudal fold skin test and a commercial gamma-interferon assay for diagnosis of bovine tuberculosis. American Journal of Veterinary Research, 56(4), 415–419.

Whipple, Diana L., Bolin, C. A., & Miller, J. M. (1996). Distribution of Lesions in Cattle Infected with Mycobacterium Bovis. Journal of Veterinary Diagnostic Investigation, 8(3), 351–354. https://doi.org/10.1177/104063879600800312

WHO. (n.d.). Retrieved from https://www.who.int/tb/strategy/en/

WHO | Tuberculosis. (n.d.). Retrieved April 24, 2017, from http://www.who.int/mediacentre/factsheets/fs104/en/

Wobeser, G. (2009). Bovine tuberculosis in Canadian wildlife: an updated history. The Canadian Veterinary Journal = La Revue Veterinaire Canadienne, 50(11), 1169–1176. Retrieved from PubMed. (20119541)

Wood, P., CORNER, L., ROTHEL, J., BALDOCK, C., JONES, S., COUSINS, D., … TWEDDLE, N. (1991). Field comparison of the interferon-gamma assay and the intradermal tuberculin test for the diagnosis of bovine tuberculosis. Australian Veterinary Journal, 68(9), 286–290. https://doi.org/10.1111/j.1751-0813.1991.tb03254.x

Wood, P. R., Corner, L. A., & Plackett, P. (1990). Development of a simple, rapid in vitro cellular assay for bovine tuberculosis based on the production of gamma interferon. Research in Veterinary Science, 49(1), 46–49.

YOKOMIZO, Y., YUGI, H., & MERKAL, R. S. (1985). A Method for Avoiding False-Positive Reactions in an Enzyme-Linked Immunosorbent Assay (ELISA) for the Diagnosis of Bovine Paratuberculosis. The Japanese Journal of Veterinary Science, 47(1), 111–119. https://doi.org/10.1292/jvms1939.47.111

Zanardi, G., Boniotti, M. B., Gaffuri, A., Casto, B., Zanoni, M., & Pacciarini, M. L. (2013). Tuberculosis transmission by Mycobacterium bovis in a mixed cattle and goat herd. Research in Veterinary Science, 95(2), 430–433. https://doi.org/10.1016/j.rvsc.2013.04.019

Zinsstag, J., Schelling, E., Roth, F., & Kazwala, R. (2008). Economics of Bovine Tuberculosis. In Mycobacterium Bovis Infection in Animals and Humans (pp. 68–83). https://doi.org/10.1002/9780470344538.ch9

Appendix A: Supplementary material for modeling the accuracy of three in-vitro bovine tuberculosis tests using a Bayesian approach.

Supplementary table S1. References for the elaboration of prior distributions for sensitivity (Se) and specificity (Sp) for intradermal (CFT, CFT-CCT) and in-vitro (IGRAb, IGRAc, ELISA) diagnostic tests evaluated**.**

Test	Antigen	Cutoff	Se	95CI		Sp	95CI		Characteristics	Reference (et al.)	Year	Origin
CFT		Any	76	56	89	100	92	100	Meta-analisis	Nuñez-Garcia	2018	UK & Ireland
		palpable	85.7			92.6			Meta-analysis	Farnham	2011	USA
		increase	83.33	51.59	97.91				Study	Norby	2004	USA
			80.4						Study	Whipple	1995	USA
			82			96			Review	USDA-APHIS	1992	USA
			65.6	56.6	73.9				Study	Wood	1991	Australia
			81.8			96.3			Study	Francis	1978	Australia
CFT-CCT		>4mm	50	26	78	100	99	100	Meta-analisis	Nuñez-Garcia	2018	UK & Ireland
			53	46	62				Study	VanderWaal	2017	Uruguay
			55.1-93.5			88.8-100			Review	Vordemeier	2006	Global
			75	42.81	94.51				Study	Norby	2004	USA
IGRA	PPDb-PPDa (IGRAb)	0.1	60.7	48	72				Study	Casal	2014	Spain
			83.5	73.6	91.6	90.4	89.1	92.7	Study	Alvarez	2012	Spain
			87.6	73	100	96.6	85	99.6	Review	de la Rua Domenech	2006	Global
			88			95			Study	Gormley	2006	Ireland
			66			84			Study	Aagaard	2006	Global
			85	72	90	93	89	96	Study	Ryan	2000	New Zealand
IGRA	Peptide - Cocktail (IGRAc)	0.1	78	60	90	99	99	100	Meta-analisis	Nuñez-Garcia	2018	UK & Ireland
			80			100			Study	Flores-Villalba	2012	Mexico
			85	73	94	97	94	100	Study	Aagaard	2006	Global
ELISA	MPB83-MPB70	>0.3	61.1	33.1	84.6	85.4	81.7	88.8	Study	Al-Mouqatea	2018	Kuwait
			57.1	44	69	100			Study	Casal	2012	Spain
			18.1			96.4			Study	Wood	1992	Australia
			61.9	30	96.7	98.2	93.8	100	Review	Waters	2012	UK,Ireland,NZ,USA

Supplementary table S2**.** Results from the sensitivity analyses using uniform distributed priors for sensitivity (Se), specificity (Sp), and Prevalence (prev) for each bTB-diagnostic test evaluated. Reference values indicate posterior median results and posterior probability intervals (LowPPI, high PPI) for the model including informative priors.

STUDY	Expert opinion prevalence priors (Models LCA-a)										
	IGRAb & CAUDAL FOLD TEST										
	Test	**Parameter**	**Prior**	**Median**	**LowPPI**	**high PPI**	**Diff in median**	**Percentage**	**Reference**	**LowPPI**	**High_PPI**
	IGRAb	Se	Table 2	0.75	0.59	0.92	0.00	-0.05	**0.75**	0.59	0.92
		Sp	Table 2	0.90	0.77	0.97	0.00	-0.17	**0.90**	0.78	0.97
	CFT	Se	dunif(0,1)	0.71	0.52	0.92	-0.02	2.79	**0.73**	0.57	0.89
		SP	Table 2	0.76	0.57	0.95	-0.01	1.42	**0.77**	0.59	0.95
		prev	Table 2	0.51	0.34	0.69	0.00	-0.35	**0.51**	0.34	0.68
		DIC		19.70			0.30	-1.55	**19.40**		
	IGRAb	Se	Table 2	0.78	0.60	0.93	0.03	-4.09			
		Sp	Table 2	0.90	0.79	0.97	0.00	-0.22			
	CFT	Se	Table 2	0.71	0.54	0.88	-0.02	2.84			
1		SP	dunif(0,1)	0.70	0.51	0.95	-0.07	8.85			
		prev	Table 2	0.49	0.33	0.67	-0.02	3.87			
		DIC		19.70							
	IGRAb	Se	dunif(0,1)	0.72	0.53	0.94	-0.04	5.05			
		Sp	Table 2	0.89	0.75	0.97	-0.01	0.83			
	CFT	Se	Table 2	0.74	0.56	0.91	0.00	-0.28			
		SP	Table 2	0.78	0.58	0.96	0.01	-1.72			
		prev	Table 2	0.52	0.33	0.71	0.01	-2.04			
		DIC		19.80							
	IGRAb	Se	Table 2	0.69	0.50	0.90	-0.06	8.39			
		Sp	dunif(0,1)	0.74	0.55	0.93	-0.16	17.88			

	CFT	Se	Table 2	0.79	0.58	0.94	0.06	-7.63
		SP	Table 2	0.76	0.55	0.96	-0.01	1.33
		prev	Table 2	0.45	0.21	0.65	-0.06	12.29
		DIC		19.70				
	IGRAb	Se	Table 2	0.72	0.56	0.90	-0.03	4.24
		Sp	Table 2	0.91	0.78	0.98	0.01	-1.13
	CFT	Se	Table 2	0.72	0.56	0.88	-0.01	1.52
		SP	Table 2	0.80	0.60	0.97	0.03	-3.81
		prev	dunif(0,1)	0.56	0.37	0.76	0.05	-10.17
		DIC		19.50				

Expert opinion prevalence priors (Models LCA-a)										
IGRAb & COMPARATIVE CERVICAL TEST										
Test	**Parameter**	**Prior**	**Median**	**LowPPI**	**high PPI**	**Diff in median**	**Percentage**	**Reference**	**LowPPI**	**High_PPI**
IGRAb	Se	Table 2	0.78	0.62	0.90	0.00	-0.38	**0.78**	0.63	0.90
	Sp	Table 2	0.90	0.74	0.98	-0.02	-1.71	**0.91**	0.79	0.98
CCT	Se	dunif(0,1)	0.56	0.38	0.83	0.02	4.51	**0.53**	0.46	0.61
	SP	Table 2	0.96	0.93	0.98	0.00	0.02	**0.96**	0.93	0.98
	prev	Table 2	0.49	0.31	0.65	-0.02	-3.33	**0.50**	0.37	0.63
	DIC		19.10					**18.10**		
IGRAb	Se	Table 2	0.81	0.65	0.93	0.03	3.83			
	Sp	Table 2	0.91	0.79	0.98	0.00	-0.42			
CCT	Se	Table 2	0.53	0.45	0.60	-0.01	-1.20			
	SP	dunif(0,1)	0.90	0.77	0.99	-0.06	-6.31			
	prev	Table 2	0.48	0.34	0.62	-0.03	-5.39			
	DIC		18.60							
IGRAb	Se	dunif(0,1)	0.76	0.56	0.91	-0.02	-2.78			
	Sp	Table 2	0.91	0.76	0.98	-0.01	-0.65			
CCT	Se	Table 2	0.53	0.45	0.61	0.00	-0.53			
	SP	Table 2	0.96	0.93	0.98	0.00	0.02			
	prev	Table 2	0.51	0.37	0.66	0.00	0.80			
	DIC		18.80							
IGRAb	Se	Table 2	0.74	0.57	0.87	-0.04	-4.61			
	Sp	dunif(0,1)	0.83	0.63	0.97	-0.09	-9.35			
CCT	Se	Table 2	0.54	0.46	0.62	0.01	0.98			
	SP	Table 2	0.96	0.93	0.98	0.00	-0.01			
	prev	Table 2	0.48	0.34	0.63	-0.02	-4.20			
	DIC		18.90							
IGRAb	Se	Table 2	0.77	0.61	0.89	-0.01	-1.54			
	Sp	Table 2	0.92	0.79	0.98	0.01	0.56			
CCT	Se	Table 2	0.53	0.45	0.60	-0.01	-1.11			
	SP	Table 2	0.96	0.93	0.98	0.00	0.06			
	prev	dunif(0,1)	0.53	0.39	0.69	0.02	4.64			
	DIC		18.30							

Expert opinion prevalence priors (Models LCA-a)

IGRAc & CAUDAL FOLD TEST

Test	Parameter	Prior	Median	LowPPI	high PPI	Diff in median	Percentage	Reference	LowPPI	High_PPI
IGRAc	Se	Table 2	0.76	0.63	0.88	0.00	-0.55	**0.76**	0.62	0.88
	Sp	Table 2	0.97	0.94	0.98	0.00	-0.03	**0.96**	0.94	0.98
CFT	Se	dunif(0,1)	0.71	0.55	0.84	-0.02	2.63	**0.72**	0.58	0.84
	SP	Table 2	0.75	0.59	0.93	-0.01	1.42	**0.76**	0.60	0.94
	prev	Table 2	0.51	0.38	0.65	0.00	0.46	**0.51**	0.38	0.65
	DIC		19.50					**19.40**		
IGRAc	Se	Table 2	0.78	0.64	0.89	0.02	-2.43			
	Sp	Table 2	0.97	0.94	0.98	0.00	-0.01			
CFT	Se	Table 2	0.71	0.57	0.83	-0.01	1.79			
	SP	dunif(0,1)	0.71	0.54	0.93	-0.05	6.73			
	prev	Table 2	0.50	0.37	0.64	-0.02	3.11			
	DIC		19.60							
IGRAc	Se	dunif(0,1)	0.79	0.52	0.93	0.03	-3.86			
	Sp	Table 2	0.97	0.94	0.98	0.01	-0.73			
CFT	Se	Table 2	0.77	0.56	0.84	0.04	-6.01			
	SP	Table 2	0.86	0.59	0.97	0.10	13.24			
	prev	Table 2	0.60	0.38	0.71	0.08	16.34			
	DIC		19.90							
IGRAc	Se	Table 2	0.73	0.57	0.87	-0.03	3.91			
	Sp	dunif(0,1)	0.79	0.62	0.95	-0.17	17.97			
CFT	Se	Table 2	0.79	0.59	0.95	0.07	-9.69			
	SP	Table 2	0.75	0.55	0.95	-0.02	2.25			
	prev	Table 2	0.42	0.20	0.61	-0.09	17.23			
	DIC		19.60							
IGRAc	Se	Table 2	0.74	0.61	0.87	-0.02	2.30			
	Sp	Table 2	0.97	0.94	0.98	0.00	-0.05			
CFT	Se	Table 2	0.72	0.58	0.84	0.00	0.28			
	SP	Table 2	0.78	0.61	0.96	0.02	-2.77			
	prev	dunif(0,1)	0.55	0.40	0.70	0.03	-6.52			
	DIC		19.40							

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Expert opinion prevalence priors (Models LCA-a)										
IGRAc & COMPARATIVE CERVICAL TEST										
Test	**Parameter**	**Prior**	**Median**	**LowPPI**	**high PPI**	**Diff in median**	**Percentage**	**Reference**	**LowPPI**	**High_PPI**
IGRAc	Se	Table 2	0.76	0.64	0.86	0.00	-0.26	**0.76**	0.65	0.86
	Sp	Table 2	0.97	0.93	0.99	0.00	-0.03	**0.97**	0.93	0.99
CCT	Se	dunif(0,1)	0.52	0.38	0.67	0.00	-0.76	**0.53**	0.46	0.60
	SP	Table 2	0.96	0.93	0.98	0.00	0.02	**0.96**	0.93	0.98
	prev	Table 2	0.51	0.40	0.64	0.00	0.10	**0.51**	0.40	0.63
	DIC		19.10					**17.70**		
IGRAc	Se	Table 2	0.79	0.67	0.89	0.03	3.50			
	Sp	Table 2	0.97	0.94	0.98	0.00	-0.01			
CCT	Se	Table 2	0.52	0.45	0.59	-0.01	-1.55			
	SP	dunif(0,1)	0.90	0.76	0.99	-0.07	-6.84			
	prev	Table 2	0.49	0.37	0.61	-0.03	-5.29			
	DIC		18.30							
IGRAc	Se	dunif(0,1)	0.74	0.57	0.88	-0.02	-2.51			
	Sp	Table 2	0.97	0.93	0.99	0.00	0.01			
CCT	Se	Table 2	0.53	0.45	0.60	0.00	-0.15			
	SP	Table 2	0.96	0.93	0.98	0.00	0.11			
	prev	Table 2	0.52	0.40	0.66	0.00	0.78			
	DIC		18,8							
IGRAc	Se	Table 2	0.74	0.62	0.84	-0.02	-3.12			
	Sp	dunif(0,1)	0.87	0.70	0.98	-0.10	-10.35			
CCT	Se	Table 2	0.54	0.46	0.62	0.01	2.10			
	SP	Table 2	0.96	0.93	0.98	0.00	0.01			
	prev	Table 2	0.48	0.34	0.61	-0.04	-7.17			
	DIC		18.70							
IGRAc	Se	Table 2	0.76	0.64	0.85	-0.01	-0.80			
	Sp	Table 2	0.97	0.93	0.99	0.00	0.04			
CCT	Se	Table 2	0.53	0.45	0.60	0.00	-0.55			
	SP	Table 2	0.96	0.93	0.98	0.00	0.07			
	prev	dunif(0,1)	0.53	0.41	0.67	0.02	3.66			
	DIC		17.90							

Expert opinion prevalence priors (Models LCA-a)										
ELISA 01 & CAUDAL FOLD TEST										
Test	**Parameter**	**Prior**	**Median**	**LowPPI**	**high PPI**	**Diff in median**	**Percentage**	**Reference**	**LowPPI**	**High_PPI**
ELISA	Se	Table 2	0.58	0.50	0.73	0.01	-0.88	**0.58**	0.49	0.73
	Sp	Table 2	0.93	0.86	0.98	0.00	0.31	**0.94**	0.86	0.98
CFT	Se	dunif(0,1)	0.98	0.92	1.00	0.03	-2.67	**0.95**	0.89	0.99
	SP	Table 2	0.67	0.37	0.95	0.03	-4.85	**0.64**	0.34	0.94
	prev	Table 2	0.76	0.57	0.86	-0.01	1.29	**0.77**	0.57	0.88
	DIC		19.10					**19.40**		
ELISA	Se	Table 2	0.67	0.54	0.82	0.10	16.45			
	Sp	Table 2	0.92	0.78	0.98	-0.02	2.10			
CFT	Se	Table 2	0.92	0.79	0.98	-0.03	3.27			
	SP	dunif(0,1)	0.31	0.09	0.62	-0.33	51.07			
	prev	Table 2	0.62	0.45	0.80	-0.15	19.18			
	DIC		20.00							
ELISA	Se	dunif(0,1)	0.58	0.48	0.87	0.01	-0.90			
	Sp	Table 2	0.93	0.85	0.98	-0.01	0.53			
CFT	Se	Table 2	0.95	0.87	0.99	-0.01	0.75			
	SP	Table 2	0.59	0.27	0.93	-0.05	7.59			
	prev	Table 2	0.76	0.47	0.89	-0.01	1.59			
	DIC		18.00							
ELISA	Se	Table 2	0.53	0.40	0.68	-0.05	7.85			
	Sp	dunif(0,1)	0.80	0.46	0.97	-0.14	15.03			
CFT	Se	Table 2	0.95	0.88	0.99	0.00	0.26			
	SP	Table 2	0.61	0.25	0.94	-0.03	4.73			
	prev	Table 2	0.76	0.42	0.87	-0.01	1.29			
	DIC		18.80							
ELISA	Se	Table 2	0.53	0.45	0.63	-0.05	8.23			
	Sp	Table 2	0.94	0.85	0.98	0.00	-0.37			
CFT	Se	Table 2	0.93	0.83	0.98	-0.03	2.75			
	SP	Table 2	0.81	0.48	0.97	0.18	-27.45			

	prev	dunif(0,1)	0.87	0.73	0.99	0.10	-12.56			
	DIC		19.60							

Expert opinion prevalence priors (Models LCA-a)										
ELISA 01 & COMPARATIVE CERVICAL TEST										
Test	**Parameter**	**Prior**	**Median**	**LowPPI**	**high PPI**	**Diff in median**	**Percentage**	**Reference**	**LowPPI**	**High_PPI**
ELISA	Se	Table 2	0.58	0.50	0.66	0.06	11.74	**0.52**	0.45	0.60
	Sp	Table 2	0.86	0.71	0.97	-0.06	-6.56	**0.92**	0.79	0.98
CCT	Se	dunif(0,1)	0.77	0.64	0.98	0.17	28.22	**0.60**	0.54	0.67
	SP	Table 2	0.96	0.93	0.98	0.00	0.15	**0.96**	0.93	0.98
	prev	Table 2	0.69	0.53	0.83	-0.11	-13.51	**0.80**	0.73	0.92
	DIC		22.50					**24.40**		
ELISA	Se	Table 2	0.60	0.49	0.73	0.08	14.52			
	Sp	Table 2	0.91	0.79	0.97	-0.02	-1.86			
CCT	Se	Table 2	0.56	0.48	0.63	-0.05	-8.13			
	SP	dunif(0,1)	0.48	0.18	0.74	-0.48	-49.60			
	prev	Table 2	0.69	0.52	0.85	-0.11	-13.28			
	DIC		23.00							
ELISA	Se	dunif(0,1)	0.53	0.45	0.62	0.00	0.54			
	Sp	Table 2	0.92	0.79	0.99	0.00	0.08			
CCT	Se	Table 2	0.61	0.55	0.67	0.00	0.22			
	SP	Table 2	0.96	0.93	0.98	0.00	0.01			
	prev	Table 2	0.83	0.72	0.92	0.03	3.99			
	DIC		24.90							
ELISA	Se	Table 2	0.48	0.39	0.58	-0.04	-7.65			
	Sp	dunif(0,1)	0.71	0.18	0.97	-0.22	-23.41			
CCT	Se	Table 2	0.60	0.54	0.67	0.00	-0.44			
	SP	Table 2	0.96	0.93	0.98	0.00	0.02			
	prev	Table 2	0.84	0.73	0.92	0.04	4.95			
	DIC		24.40							
ELISA	Se	Table 2	0.48	0.42	0.56	-0.04	-7.52			

	Sp	Table 2	0.93	0.79	0.98	0.00	0.15			
CCT	Se	Table 2	0.57	0.51	0.63	-0.04	-5.97			
	SP	Table 2	0.96	0.93	0.98	0.00	0.18			
	prev	dunif(0,1)	0.94	0.82	1.00	0.15	18.30			
	DIC		22.40							

CFT-estimated prevalence prior distribution (Models LCA-b)

IGRAb

Test	Parameter	Prior	Median	LowPPI	high PPI	Diff in median	Percentage	Reference	LowPPI	High_PPI
IGRAb	Se	dunif(0,1)	0.52	0.40	0.79	0.52	9.76	**0.58**	**0.43**	**0.86**
	Sp	Table 2	0.92	0.77	0.99	0.92	0.36	**0.93**	**0.78**	**0.99**
	prev	Estimated	0.83	0.53	0.98	0.83	-8.92	**0.77**	**0.48**	**0.97**
	DIC		7.20					**7.30**		
IGRAb	Se	Table 2	0.55	0.36	0.93	-0.03	-5.38			
	Sp	dunif(0,1)	0.60	0.10	0.98	-0.33	-35.10			
	prev	Estimated	0.73	0.00	0.97	-0.04	-4.78			
	DIC		7.50							
IGRAb	Se	Table 2	0.75	0.47	0.96	0.17	28.49			
	Sp	Table 2	0.92	0.75	0.99	-0.01	-1.00			
	prev	dunif(0,1)	0.54	0.31	0.92	-0.22	-29.05			
	DIC		7.10							
IGRAc										
IGRAc	Se	dunif(0,1)	0.49	0.37	0.77	-0.17	25.08	**0.66**	0.47	0.87
	Sp	Table 2	0.97	0.93	0.99	0.00	0.07	**0.97**	0.94	0.99
	prev	Estimated	0.83	0.53	0.98	0.18	-27.70	**0.65**	0.46	0.92
	DIC		7.20					**7.80**		
IGRAc	Se	Table 2	0.73	0.45	0.91	0.07	10.74			
	Sp	dunif(0,1)	0.61	0.49	0.99	-0.36	-36.85			
	prev	Estimated	0.00	0.00	0.91	-0.65	-100.00			
	DIC		7.80							
IGRAc	Se	Table 2	0.77	0.55	0.91	0.11	16.19			
	Sp	Table 2	0.97	0.93	0.99	0.00	-0.09			

	prev	dunif(0,1)	0.52	0.37	0.78	-0.13	-20.05			
	DIC		7.20							
ELISA										
Test	**Parameter**	**Prior**	**Median**	**LowPPI**	**high PPI**	**Diff in median**	**Percentage**	**Reference**	**LowPPI**	**High_PPI**
ELISA	Se	dunif(0,1)	0.54	0.44	0.79	0.00	0.26	**0.54**	0.44	0.71
	Sp	Table 2	0.93	0.78	0.99	0.00	0.11	**0.92**	0.79	0.99
	prev	Estimated	0.84	0.54	0.98	0.00	-0.24	**0.84**	0.60	0.98
	DIC		8.00					**8.40**		
ELISA	Se	Table 2	0.48	0.34	0.70	-0.06	-10.33			
	Sp	dunif(0,1)	0.57	0.05	0.98	-0.35	-38.20			
	prev	Estimated	0.83	0.00	0.98	-0.01	-0.88			
	DIC		8.00							
ELISA	Se	Table 2	0.59	0.45	0.81	0.05	8.77			
	Sp	Table 2	0.92	0.76	0.99	0.00	-0.49			
	prev	dunif(0,1)	0.75	0.47	0.98	-0.09	-10.43			
	DIC		8.00							

Appendix B: Supplementary material for modeling bTB within-herd dynamics with the use of different diagnostic strategies in high prevalence herds when depopulation is not feasible.

Figure 5.8.S1.

Each line represents the median proportion of animals in each infected compartment (Occult, Reactive.a, Reactive.b, and Infectious) after 500 iterations. True 10% (Disease) and apparent 10% (Reactive.a + Reactive.b + Infectious) prevalence of bTB per year when simulating bTB-transmission without any control strategy applied are indicated by the intercept with the horizontal line.

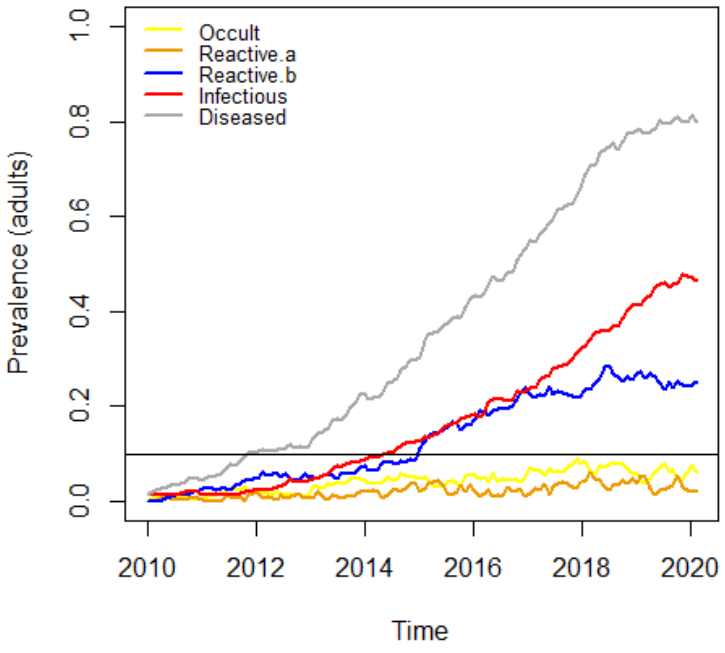
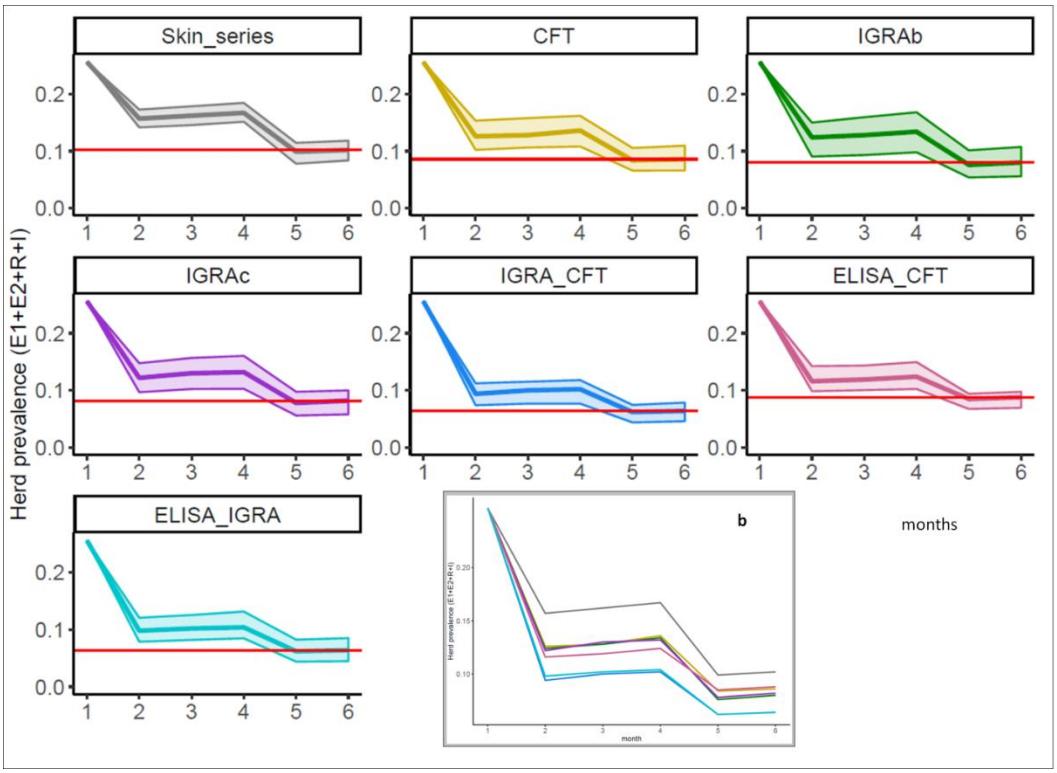
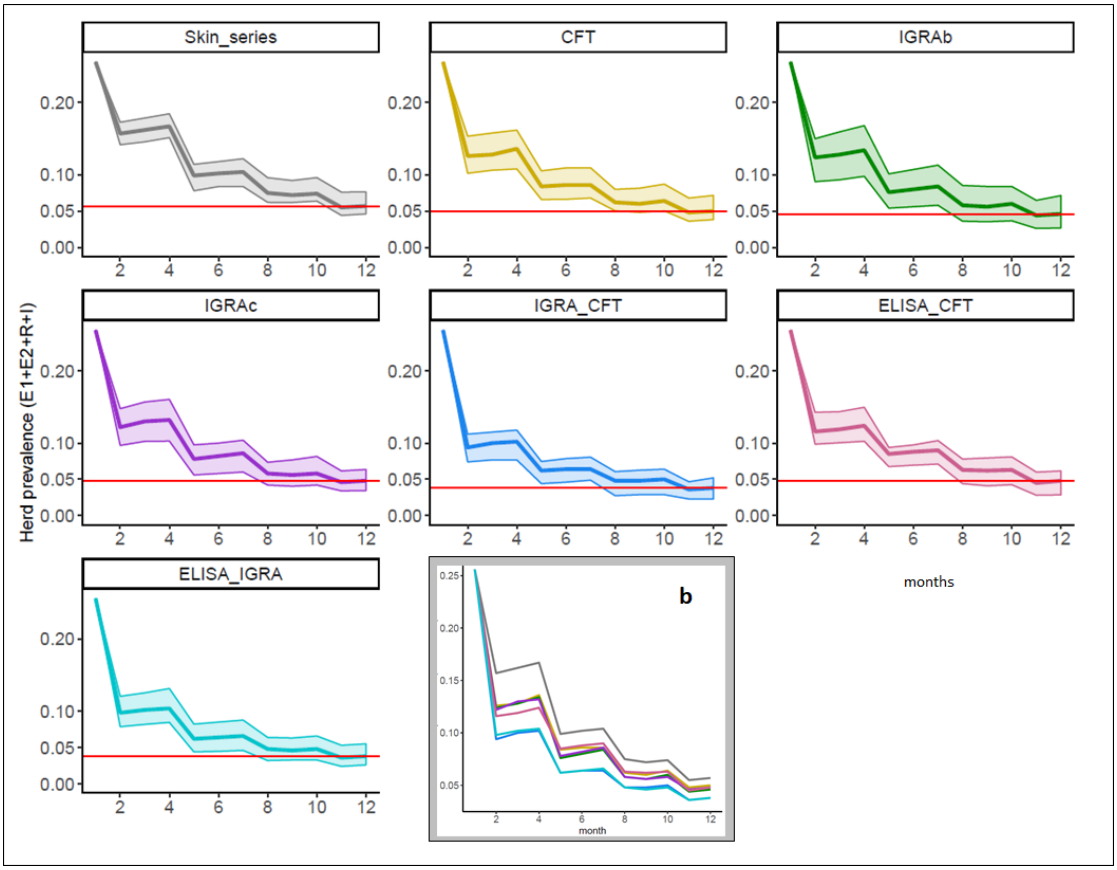


Figure 5.8.S2. The median and 95_{th} percentile of bTB-prevalence on the first six months of simulations for each independent strategy scenario, and the comparison of the median bTB-prevalence estimate for all scenarios (b). Median bTB-prevalence at the end of the year (red line) is represented for all the simulated strategies respectively.



Skin_series: CFT and Comparative Cervical tuberculin test with in the series application
CFT: Caudal Fold tuberculin test
IGRAb: Interferon-gamma release assay using PPDb-PPDa antigens
IGRAc: Interferon-gamma release assay using peptide cocktail antigens
ELISA: Commercial Enzyme-immunosorbent assay

Figure 5.8. S3. The median and 95_{th} percentile of bTB-prevalence per month on year one of simulations for each independent strategy scenario, and the comparison of the median bTB-prevalence estimate for all scenarios (b). Median bTB-prevalence at the end of the year (red line) is represented for all the simulated strategies respectively.



Skin_series: CFT and Comparative Cervical tuberculin test with in series application
CFT: Caudal Fold tuberculin test
IGRAb: Interferon-gamma release assay using PPDb-PPDa antigens
IGRAc: Interferon-gamma release assay using peptide cocktail antigens
ELISA: Commercial Enzyme-immunosorbent assay

Figure 5.8. S4. The median and 95_{th} percentile of bTB-prevalence per month on year two of simulations for each independent strategy scenario, and the comparison of the median bTB-prevalence estimate for all scenarios (b). A reference 2.5% bTB-prevalence (red line) is shown for the status quo (Skin_series), caudal fold test (CFT), interferon-gamma release assay with PPD bovis and PPD avium antigen (IGRAb), interferon-gamma release assay with peptide cocktail antigen (IGRAc), and parallel combinations for IGRAc and CFT (IGRA_CFT), ELISA and CFT (ELISA_CFT), and ELISA and IGRAc (ELISA_IGRA) respectively.

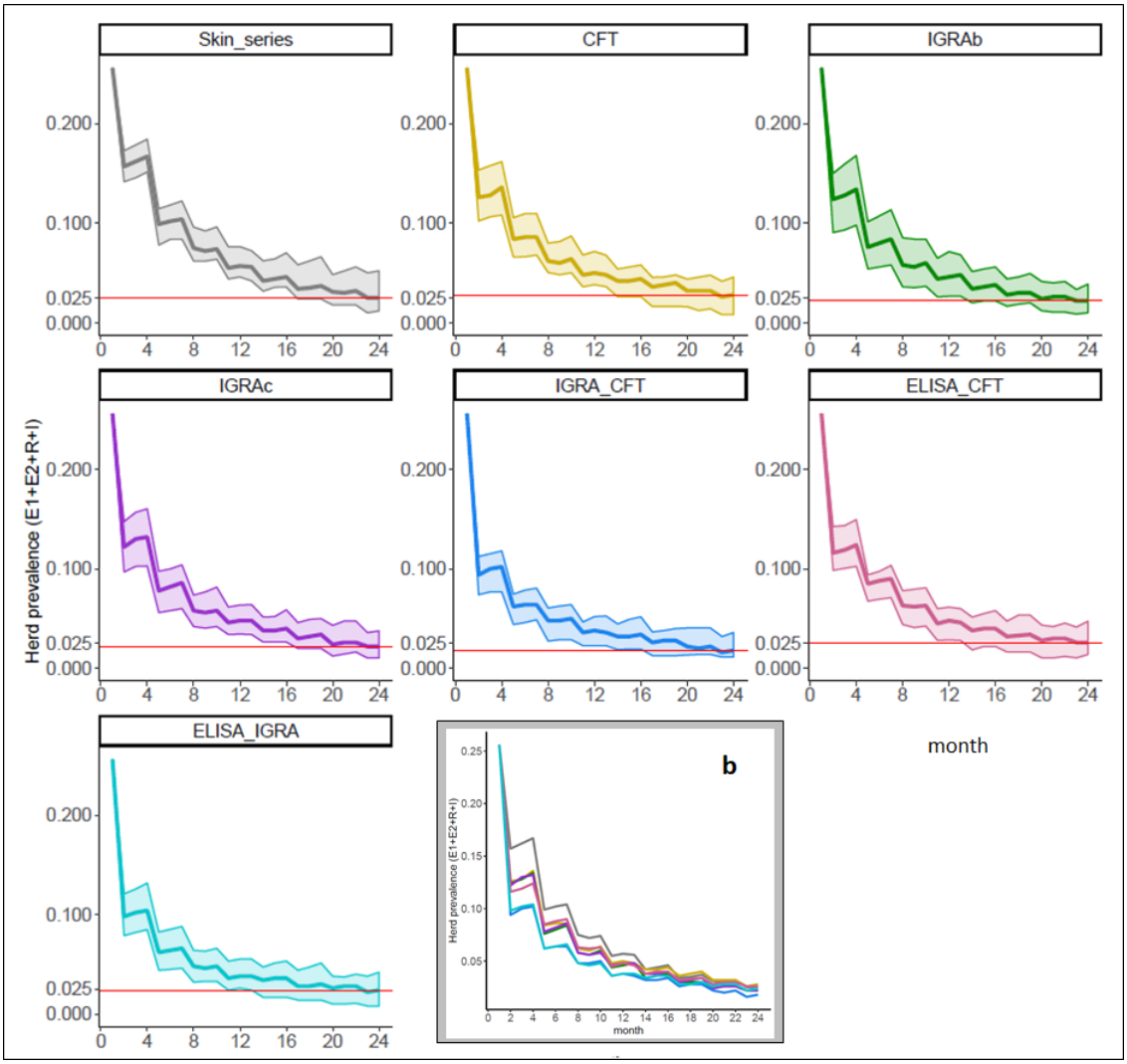

Skin_series
CFT
IGRAb
IGRAc
IGRA_CFT
ELISA_CFT
ELISA_IGRA
Herd prevalence (E1+E2+R+I)
0.200
0.100
0.025
0.000
0 4 8 12 16 20 24
month
b
0.25
0.20
0.15
0.10
0.05
0 2 4 6 8 10 12 14 16 18 20 22 24

Figure 5.8. S5. Distribution (median, 95_{th} percentile) of the 500 simulations of the predictive positive value of the for the status quo (0.Skin_series) and the six alternative control scenario in the first 6 (left), 12 (center) and 24 months (right) of testing implementation.