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Could a vaccine eradicate bovine tuberculosis in Portugal before 2025?

Mestrado em Saúde Pública

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Could a vaccine eradicate bovine tuberculosis in Portugal before 2025?

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Abstract

Introduction: Bovine tuberculosis (bTB) is a zoonotic disease, which affects cattle. In Portugal, bTB prevalence is low and the country aims to eradicate the disease by 2025. However, the presence of wildlife reservoirs and the long latent stage complicates this objective. This work aims to understand the impact of *Bacillus* Calmette-Guérin (BCG) in the control of bTB in Portugal through mathematical modelling and, whether it would be possible to eradicate the disease before 2025.

Methods: A mathematical model (Susceptible- Exposed- Infectious– Susceptible vaccinated- Exposed vaccinated- Infectious vaccinated) representing bTB transmission between cattle with the BCG vaccine was built and implemented in R. Parameters were abstracted from the literature and data from the Portuguese bTB eradication programme. The impact of BCG on bTB was simulated using different culling and vaccination rates. The final outputs were the basic reproduction number (R_0), the number of infectious animals, the bTB prevalence and the disease reduction.

Results: When vaccination is considered, at any rate, the burden of disease is similar to the model only considering test and slaughter strategy. The only difference is the number of vaccinated infectious animals, which is higher when the vaccination rate increases and there is a slight decrease in R_0. The major impact on decreasing the bTB infection burden is when the culling rate is 1 with and without vaccination.

Discussion and Conclusion: The results suggest that the vaccination will not eradicate bTB before 2025. However, more research is needed to understand the impact of BCG on bTB. Further projects should explore the stochastic effects of bTB and focus on herds in high-risk areas.

Keywords: Bovine tuberculosis, Portugal, Mathematical Model, BCG vaccine, disease eradication.

Resumo

Introdução: A tuberculose bovina (TBb) é uma doença zoonótica, que afeta maioritariamente o gado bovino. Em Portugal, a prevalência da TBb é baixa e o objetivo é erradicar a doença até 2025. Contudo, os reservatórios da doença em animais selvagens e o longo período de latência dificultam a sua concretização. Este trabalho tem como objetivo compreender o impacto da *Bacillus* Calmette-Guérin (BCG) no controlo desta doença em Portugal através de modelação matemática e se é possível erradicá-la antes de 2025.

Métodos: Foi construído e implementado em R um modelo matemático (Suscetível- Exposto- Infecioso- Suscetível vacinado- Exposto vacinado- Infecioso vacinado) que representa a transmissão de TBb entre o gado bovino com a implementação da BCG. Os parâmetros foram extraídos da literatura ou da base de dados do programa de erradicação em Portugal. O impacto da vacinação foi estimado para várias taxas de abate e vacinação. Os resultados obtidos foram o número básico de reprodução (R_0), o número de animais infeciosos, a prevalência de TBb e a redução da doença.

Resultados: Quando a vacinação é considerada, qualquer que seja a taxa, a infeção de TBb é igual aquando apenas a estratégia de teste e abate é considerada. A única diferença é o número de animais infeciosos vacinados, que é superior quando a taxa de vacinação aumenta e o pequeno decréscimo no R_0. O maior impacto no decréscimo da TBb é quando a taxa de abate é 1 com ou sem vacinação.

Discussão e Conclusão: Os resultados sugerem que a vacinação não irá erradicar TBb antes de 2025. Contudo, mais investigação é necessária para analisar o impacto da BCG. Trabalhos futuros deverão explorar os efeitos estocásticos de TBb e focar em manadas das áreas de maior risco de infeção.

Palavras-chave: Tuberculose bovina, Portugal, Modelo matemático, vacina da BCG, erradicação da doença.

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List of abbreviations and acronyms

bTB - Bovine tuberculosis

BCG - *Bacillus* Calmette-Guérin

R_0 - Basic reproduction number

DIVA - Differentiating Infected from Vaccinated Animals

CMI - Cell-medicated immune

IFN-ɣ - Interferon-gamma

OIE - World Organisation for Animal Health

WHO - World Health Organisation

UK - United Kingdom

LTV - Lisbon and Tagus Valley

DGAV - Direção-Geral da Alimentação e Veterinária (Directorate-General for Health and Food Safety)

PPD - Purified Protein Derivative

SITT - Single Intradermal Tuberculin Test

SICTT - Single Intradermal Comparative Tuberculin Test

ELISA - Enzyme-Linked Immunosorbent Assay

PCR - Polymerase Chain Reaction

1 Introduction

Bovine tuberculosis (bTB) is a disease of major concern for animal health (1). It is a chronic bacterial disease mainly due to *Mycobacterium bovis*, which is host-adapted to cattle (1). However, it can be transmitted to other domesticated animals, wild animals and even humans through direct or indirect contact, such as eating raw meat and drinking unpasteurized milk from infected animals (1). The symptoms of an infection with *M. bovis* in animals are pneumonia, weight loss and even death due to nodules formed in the lymph nodes, lungs and other tissues (1).

M. bovis is the most common cause of zoonotic tuberculosis, i.e., human tuberculosis caused by transmission from animals (mostly cattle) (1). While tuberculosis transmission within humans is mainly due to *Mycobacterium tuberculosis,* zoonotic tuberculosis also needs to be considered to control human tuberculosis (1). When comparing infection in humans by *M. tuberculosis* and *M. bovis*, it is not possible to clinically differentiate both infections (1). This means that zoonotic tuberculosis is probably underdiagnosed (1).

Overall, bTB is a disease with complex epidemiology, which is endemic in many geographical areas in domestic cattle (2,3). It has a major negative impact on the economy due to the reduction of milk and meat production and it is a barrier to the international trade of animals and their products, due to the infected animals' slaughter and prohibition of cattle movement from infected herds (2,3). Prevention, surveillance, and eradication strategies are required to control bTB, and they are applied in many countries (1,4,5). Also, in most of them, treatment and vaccination are forbidden because *M. bovis* is resistant to many of the existing antibiotics and the only vaccine with proven protection against this pathogen is the *Bacillus* Calmette-Guérin (BCG) (1,4,5). This vaccine interferes with the standard diagnostic method used (1,4,5). Therefore, the most common control strategy is the test and slaughter which tests all animals aged more than 6 weeks and slaughter the positive ones (1,4,5).

Portugal is one of the countries with an eradication program, which consists of test, slaughter and movement control intending to eradicate bTB until 2025 (6). However, this disease persists and one of the reasons is the contact of cattle with infected wild animals (1). One possible solution to diminish the impact of wildlife spill overs could be the cattle vaccination with BCG, but vaccination in the European Union is forbidden (7). Thus, the use of this vaccine would require the development of a Differentiate Infected from Vaccinated Animals (DIVA) test, which is capable of distinguishing vaccinated from infected animals (8–10). Alternatively, a new vaccine with the same or better efficacy, not interfering with the tuberculin skin test would be an option (8–10).

As the BCG vaccine is forbidden in many countries the literature assessing its contribution to bTB eradication is scarce. Therefore, my project assessed if a vaccine could help the eradication of this disease in the Portuguese mainland after 2025. To this end, a mathematical model representing bTB in Portugal with the control measures already implemented and including vaccination was developed. Thus, the model simulated how the number of infected animals would be with a vaccine implemented.

This work includes 6 sections in addition to the current one. In the background, the literature is reviewed, and aspects relevant to bTB are described, including the bovine and zoonotic tuberculosis impact either on the animals or human's health. Also, the natural disease history and the epidemiology of bTB in the world and Portugal is provided. Additionally, the control measures, vaccination against bTB and the role of mathematical models in this context are explained. Finally, the objectives of this work are presented. In the methods section, information on the methods used to address the objectives of this work is provided. It starts with a broad explanation of mathematical models and then the model implementation. In the results section, the results are presented graphically and numerically and in the discussion section, they are analysed concerning the objectives and existing literature. In the conclusion, the findings are summarised, and in the recommendation section, there are recommendations for the future.

2 Background

2.1 Bovine and zoonotic tuberculosis

Bovine tuberculosis is a cattle disease, which is a chronic or subacute disease caused by bacteria from the *Mycobacterium tuberculosis* complex, mainly by *Mycobacterium bovis* but also by *Mycobacterium caprae* (1). This disease has a variable rate of progression but normally the course of bTB is slow, taking sometimes years to kill an infected animal (1).

After infection, nonvascular nodular granulomas, known as tubercles, can develop (1). These lesions occur normally in the lungs and the retropharyngeal, bronchial, and mediastinal lymph nodes (11). The infected animals might take years to manifest the clinical signs and often the disease is subclinical (1). The symptoms include weakness, weight loss, fluctuating fever, dyspnoea, intermittent cough, pneumonia, diarrhoea and enlarged lymph nodes (1).

As abovementioned*, M. bovis* is host-adapted to cattle (1). However, it can be transmitted to other domesticated animals, wild animals and even humans through direct or indirect contact, such as eating raw meat and drinking unpasteurized milk from infected animals (see section 2.2.1) (1). Zoonotic tuberculosis occurs when tuberculosis is transmitted from animals, mostly from domestic animals (i.e., cattle), to humans and it is mainly due to *M. bovis* (1). Even though zoonotic tuberculosis is a health concern, the majority of tuberculosis cases within humans is mainly due to *Mycobacterium tuberculosis,* with a quarter of the world's population at risk of being infected by this bacterium (12). When comparing infection by *M. tuberculosis* and *M. bovis* in humans, it is not possible to clinically differentiate both infections, which means that zoonotic tuberculosis is under-diagnosed (1). In some countries, mostly in Africa and South-East Asia, where bTB is not controlled among animals and pasteurization of milk is less regulated, zoonotic tuberculosis due to *M. bovis* may be responsible for 10% of human tuberculosis cases (1). Nevertheless, the data is limited and the correct number of cases remains uncertain (1).

Furthermore, *M. bovis* is resistant to pyrazinamide, one of the first-line antimicrobials commonly used to treat tuberculosis caused by *M. tuberculosis* (2). Therefore, to initiate treatment against zoonotic tuberculosis is essential to do a drug susceptibility test, to not create resistance to other tuberculosis antibiotics, a huge threat to human global health (2). Fighting zoonotic tuberculosis requires primarily fighting the core problem - bTB in cattle (2). If the disease is eradicated in cattle, the probability of transmission to humans is lower, because bovines are their major bTB transmission vector (2).

Also, bTB has a major negative impact on the economy due to the reduction of milk and meat production, the expenses associated with animal slaughter and bTB are a barrier to the international trade of animals and animal products (2,3). Therefore, controlling bTB could generate improvements for human and animal health, and for the economy (2,3).

2.2 Natural disease history

2.2.1 Aetiology and Transmission

The *M. bovis* and *M. tuberculosis* are mycobacteria from the *Mycobacterium tuberculosis complex*, which are aerobically restricted, do not release spores and are gram-positive (3,13). Their cellular wall has a lipidic component and mycolic acid, which makes this pathogen resistant to most disinfectants and acids (3,13). Unlike *M. tuberculosis, M. bovis* is naturally resistant to pyrazinamide, an antibiotic used to combat tuberculosis in humans (1). Its survival in the environment is influenced by temperature, humidity, ultraviolet radiation, and sunlight exposure: in a warm and humid environment with light protection, this pathogen can be viable for several weeks in water, soil, hay and corn (3,13).

Research on bTB dates back from the 19th century and it was crucial to understand the *M. bovis* transmission to humans (14). In 1898, Theobald Smith reported that human tuberculosis and bTB were caused by different bacteria (14). In 1911, a commission of tuberculosis experts concluded that bTB could be transmitted from animals to humans (14). There is evidence of this transmission between 1901 and 1932, where 91% of cervical lymph nodes and 28% of meningeal TB cases in children were caused by *M. bovis*, demonstrating the zoonotic potential of this disease (14). Even before these findings, some researchers have linked human tuberculosis with contact with infected animals and the consumption of non-pasteurized milk, making this the most probable route of transmission (14). Such recognition led to the implementation of milk pasteurisation and boiling, decreasing the impact of the digestive route of infection (14). However, airborne infection kept being an important route of transmission, with individuals in jobs with close contact with animals experiencing high levels of infection, demonstrating the importance of controlling this route of transmission (14).

Pathogen characteristics are important for its transmission among animals and humans. Overall, bTB is a disease with a complex epidemiological pattern, which is endemic in domestic cattle in many geographical areas (1,2). A simple scheme of transmission of this disease is portrayed in Figure 1. The transmission dynamics within cattle is through direct contact with an infected animal or contaminated environment, i.e., pasture (1,2). Also, there are different routes of transmission between cattle. For instance, calves can

be infected by ingesting colostrum or milk from infected cows (1,15). However, the main route of infection within cattle herds is airborne, which means transmission occurs through droplets (1). The same happens within wild animals (1,2). In addition, there are interactions between wild animals and cattle because some herds are in contact areas with certain animals' habitats, which leads to disease transmission (1,2).

When the transmission is from animals to humans, mainly from cattle, people can get infected through direct contact with infected animals in specific jobs, such as farmworkers, slaughter workers and veterinaries, making this disease an occupational hazard (1). Also, people can get infected through indirect contact, which has been reduced with the existing food hygiene measures, even though it can occur with the consumption of unpasteurized dairy products or undercooked or raw meat from infected animals (1,2). Lastly, transmission from wild animals to humans occurs mainly while hunting when there is contact with infected carcasses (1,2).
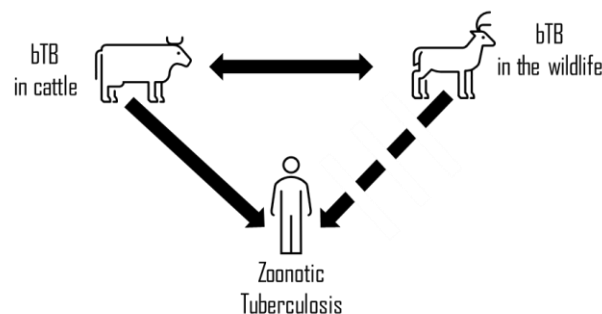
Figure 1: Bovine tuberculosis transmission among cattle, wild animals, and humans. The full lines represent common sources of infection while the dashed lines are rarer. Source: Adapted from WHO, OIE (1,2).

2.2.2 Pathogenesis and Immune response

Bovine tuberculosis is a chronic or subacute disease (1,11,15). It has a slow progression with a long latent stage (1,11,15). In this latent stage, the animals are not infectious, and they remain asymptomatic, making detection at this stage harder (1,11,15).

The primary lesions from bTB are related to the route of transmission responsible for the infection (15). As the most common transmission route in cattle is through aerosols, the infection appears mostly in the nasopharynx and lower respiratory tract, lungs and draining lymph nodes (1,11,15). These lesions are normally granulomas, and they can be localised or disseminated to other tissues and organs (15). When the lung is the most affected organ it can be expressed by cough, dyspnoea, and pneumonia (1). When the digestive tract is involved, there is diarrhoea or constipation (11). At more advanced stages, there is an enlargement of lymph nodes which can obstruct air passages, the alimentary tract, or blood vessels (11). In terminal stages, acute respiratory distress

might occur (11). However, clinical signs are not always presented, even in advanced cases (1).

The knowledge of how immune response is involved in disease development is essential to the basic understanding of bTB (16). This relates not only to the ability to follow disease progression, but also to know how to improve basic tools of disease eradication, such as diagnostic tests and vaccines, which are dependent on immune responses (16).

Following *Mycobacterium* infection, the immunologic response occurs, which consists of both cell-mediated immune (CMI) response and humoral response, where CMI dominates (16,17). The CMI is an immune response that does not involve antibodies but rather involves macrophages and natural killer cells that destroy the pathogens (18). These cells also produce cytokines that stimulate other cells involved in the adaptative and innate immune response (18).

CMI response is an early robust response (two-three weeks after challenge with *M. bovis*) which is essential in the prevention of visible lesions and their dissemination (19). This response produces interferon-γ (IFN-γ), a key cytokine in the mycobacterial combat, including activation of the microbicidal mechanisms of macrophages (17,19).

The other type of immune response is humoral, which consists of antibodies produced by B cells (20). These destroy pathogens and prevent the spread of intracellular infections (20). For bTB, the humoral response is considered supportive rather than essential and it appears at a more advanced stage of infection, starting two-four weeks after infection (19,21,22).

The understanding of the immune response is an important way of acknowledging the disease's progression. As abovementioned, the disease has normally a long progression and takes several years to be fatal. This makes the immunological response crucial to diagnose the disease as it is not possible to rely on the clinical signs.

2.3 Epidemiology

2.3.1 World Epidemiology

Bovine tuberculosis is found all around the globe. The prevalence varies due to differences in local livestock management, wildlife reservoirs and existing disease control measures (1). Worldwide, the highest prevalence of this disease is in Africa and Asia (1). In these locations, bTB is a public health concern because of its zoonotic potential and the difficulties in the implementation of control measures (1). These continents include several low- and middle-income countries where there is no budget for eradication programmes (10).

Between January 2017 and June 2018 from the 188 countries which report their bTB situation to the World Organisation for Animal Health (OIE), only 82 countries reported bTB cases (23). Twenty-nine countries reported the presence of bTB in livestock and wildlife, two reported bTB presence only in wildlife and 51 reported only to have cases in livestock (Figure 2) (23).
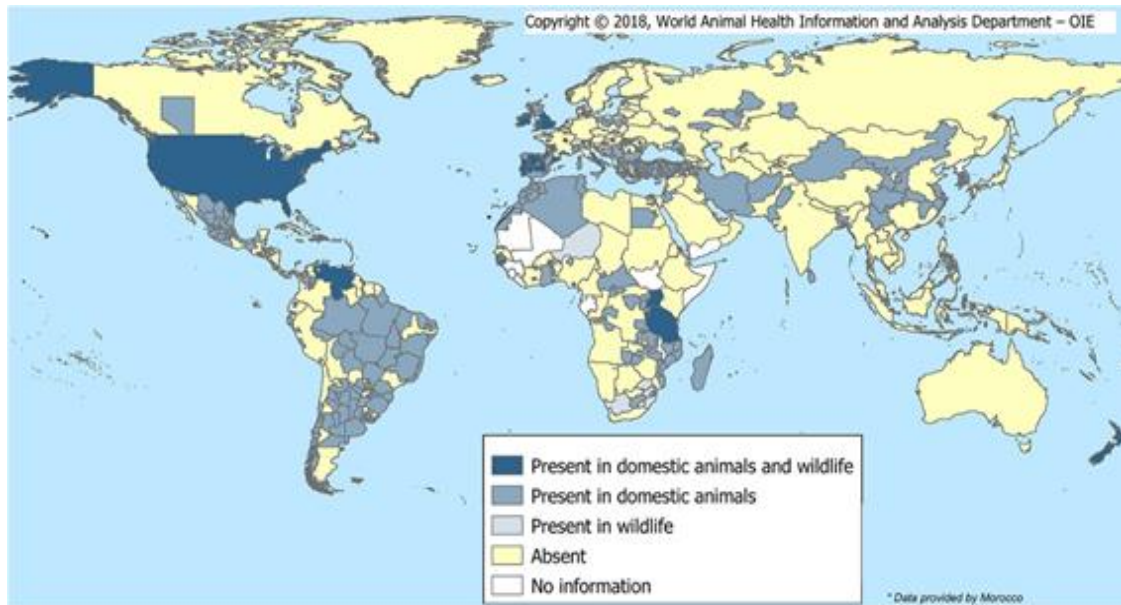
Figure 2: Worldwide distribution of bovine tuberculosis in 2017 and the first semester of 2018. Source: World Organisation for Animal Health (23).

In the countries where bTB is endemic, there is a higher probability of zoonotic tuberculosis (12). Table 1 provides the number of new cases of zoonotic tuberculosis and deaths due to this disease in 2019 by the World Health Organisation (WHO) region, mainly caused by *M. bovis* (12). While estimates exist, for both the incidence and mortality due to zoonotic tuberculosis, uncertainty intervals are wide, due to the absence of routine reporting in most countries where bTB is endemic (12). To improve zoonotic tuberculosis-related estimates, surveillance systems strengthening for both human and animal disease in most countries is required (2).

Table 1: Estimated incidence and mortality due to zoonotic tuberculosis for WHO regions and globally, 2019.

WHO Region	Number of incident cases		Number of deaths	
	Best Estimate	Uncertainty Interval	Best Estimate	Uncertainty Interval
Africa	68900	18500-152000	8440	2220-18700
South-East Asia	43400	11200-96900	2020	548-4440
Eastern Mediterranean	8190	2110-18300	604	161-1340
Western Pacific	1800	4720-40000	270	73-594
Europe	986	263-2180	65	18-143
The Americas	870	236-1910	42	11-92
Global	140000	69800-235 000	11400	4470-21600

Source: WHO (12).

In Europe, the epidemiological situation regarding bTB is heterogeneous (24). Some countries are free of bTB, while others do not have any disease-free region, such as Bulgaria, Croatia, Cyprus, Greece, Ireland, Malta and Romania (24). On the other hand, other countries have some regions officially free of bTB, such as Italy, Portugal (Algarve), Spain and the United Kingdom (UK) (24).

For a country to be considered officially free of this disease all cattle should be reported, all slaughtered cattle need to be analysed post-mortem and the country needs to have 99.9% of herds bTB-free for 6 years in a row (6,25). In some countries, its elimination is a challenge because of its persistence in wildlife, as is the case of wild boar and deer in the Iberian Peninsula (1).

2.3.2 Epidemiology in Portugal

In Portugal, bTB is monitored and the herd bTB prevalence in the mainland has been stable since 2012 (26). In 2019, according to the European Commission estimates, in the mainland, the herd prevalence was 0.44% and the herd incidence was 0.34%, where the majority of the positive herds were new positives (26).

Nevertheless, on the mainland, there are regional differences. As presented in Figure 3, Centro and Alentejo regions have more cases of bTB, representing 70.87% of the herds with confirmed infection with *M. bovis* (26). This is due to the existence of several contact areas with wildlife (deer and wild boars), which are a relevant source of tuberculosis infection (6,26). Lisbon and Tagus Valley (LTV) region has few herds and when there is an increase in positivity it leads to a significant increase in the prevalence in that region (26). Also, there is only a region of the mainland that is officially free of bTB, Algarve (since 2012) (6,26).
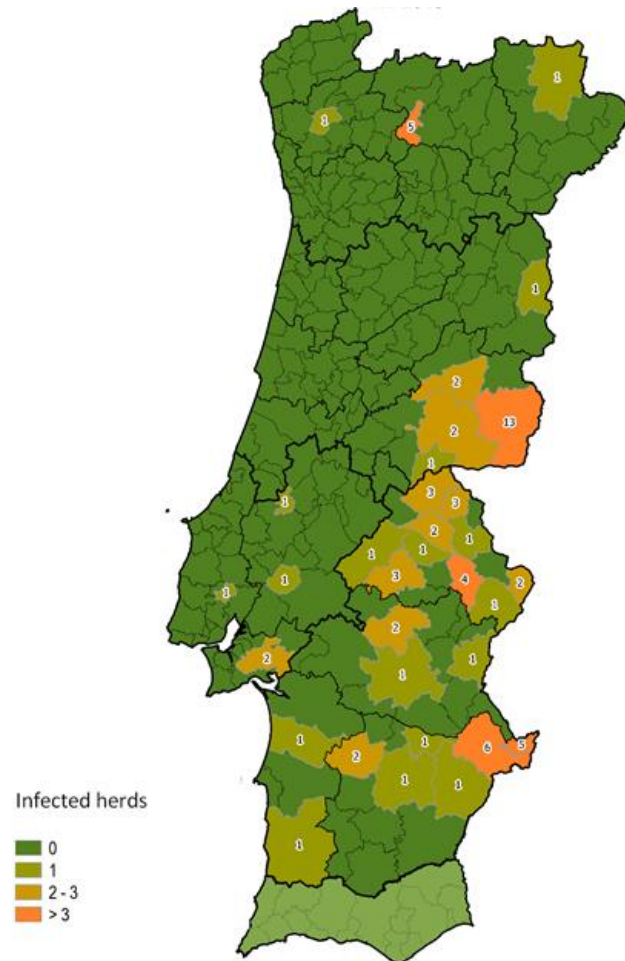

Figure 3: Infected herds distribution in 2019. Source: Annex of Eradication: Final report for Bovine Tuberculosis 2019, access given by DGAV (26).

Analysing the bTB and herd prevalence since 2012 (when Algarve turned free of bTB), there was a reduction of this disease, from 0.34% of positive holdings in 2013 to 0.24% in 2018 (Figure 4) (6,26). Unfortunately, in 2019 there was an increase in bTB positivity, which can be justified by the increase in the number of tested herds and the possible contact with wild animals (6,26). This means that implemented control measures are important, but not enough to control the wildlife infection (26,27).
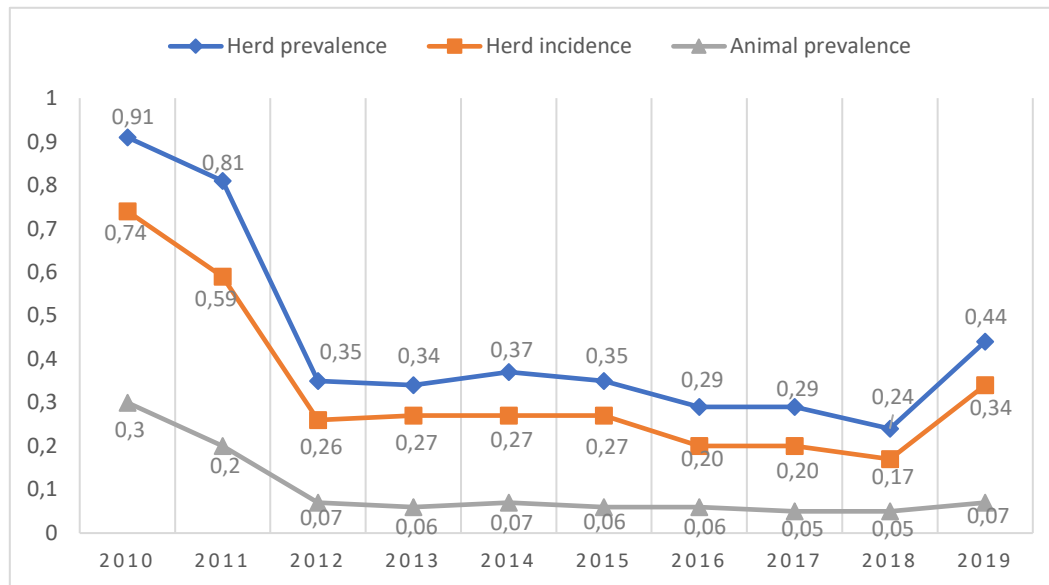
Figure 4: Bovine Tuberculosis in Portugal: Herd prevalence and incidence and Animal prevalence from 2010 to 2019. Source: Adapted from the Annex of Eradication: Final report for Bovine Tuberculosis 2019, access given by DGAV and Animal Health report 2010-2016 (DGAV) (26,27).

As presented by the data the bTB herd and animal prevalence is low in Portugal. However, in the last 5 years, it has been in a steady state, with a herd prevalence between 0.35% to 0.44% (Figure 4) (26,27).

2.4 Control measures

Control measures refer to the strategies to control the spread of bTB and possibly attain eradication in cattle. These strategies focus on ways to avoid the routes of transmission (28). Such measures are intended to interfere within cattle, between cattle and humans, and between wildlife and cattle transmission (28).

The strategies available to prevent bTB within cattle are normally implemented in eradication programs which consist of post-mortem meat inspection, intensive surveillance, systematic individual testing of cattle and slaughtering of infected animals, and monitoring movement between herds with testing (1,28). When a herd has animals that test positive, these animals are slaughtered, and their herds are tested more frequently (see section 2.4.1 for further details) (28). When considering alternative control strategies the more effective is the whole-herd slaughter (when there is a positive

case) or additional national testing (28). However, they imply a great negative economic impact on the farmers (28).

Cattle to human transmission occurs through direct contact with infected animals, and meat and milk consumption (1,28). Thus, post-mortem meat inspection prevents unsafe meat from entering the food chain and allows veterinary services to trace back the herd of origin of the infected animal (1,28). Furthermore, the bacteria is killed through milk pasteurisation, which prevents the spread of disease to humans (1,28). Similarly, good hygiene practices during slaughter and meat production can reduce the risk of direct transmission to humans (1).

The existing measures to prevent transmission from wild animals to domestic animals include vaccination of wild animals with an oral route vaccine (29). Vaccination should prevent the spread of infection to other animals (29). Recent studies in multiple wildlife species have shown that the BCG vaccine can fulfil these requirements and provide protection against bTB (29). Also, other strategies are the selective and non-selective culling of wild animals next to bovine holdings (29).

Only at the end of the 20th century, most European countries introduced national control programmes for bTB to eradicate this disease (1,4,5). These programmes consist of three main components: prevention, surveillance, and eradication (1,4,5). Nevertheless, in most countries, the treatment of bTB in cattle is not permitted, because of the doses and duration of treatment required, the high cost of medications, and the potential risk of additional antimicrobial resistance development (1,4,5). Furthermore, BCG vaccination is not used as a preventive measure in cattle, as it is used in humans, due to the potential interference with the tuberculin test (1).

2.4.1 In Portugal

The Portuguese eradication program started in 1991, and it is co-financed by the European Commission (6). It aims to eradicate bTB by 2025, through the reduction of incidence and prevalence of this disease in cattle (6). This program classifies herds by status and defines testing rules and animals' movement control according to their status (6).

The eradication program is the responsibility of the Directorate-General for Health and Food Safety (DGAV) (6). It is implemented in mainland Portugal, except for Algarve, which is free from bTB since 2012, the Autonomous Region of the Azores and the Autonomous Region of Madeira (6). In the mainland, the eradication program is controlled by four regional directorates (Norte, Centro, LVT, Alentejo) (6). They are responsible for the herd's status definition, epidemiologic research, slaughterhouse

inspection and supervision of animals identification and testing (6). In the mainland, the programme covers all bovines over 6 weeks of age (6). The herds are divided into 4 statuses: T3, T3S, T2, T2.1, where the tuberculosis-free herds have the T3 status. In holdings that are not officially tuberculosis-free, animals are subject to testing until their herd achieves disease-free status (6). Table 2 describes the status and measures applied (6). Figure 5 depicts the transition between statuses (6).

Table 2: Differences between the holding health status in Portugal, regarding status transition and allowed movements.

|	Holding classification status			
	T3	**T3S**	**T2.1**	**T2**
Definition	Officially disease-free.	Suspension of T3 status when there is a positive test or suspected lesions are detected or epidemiological inquiry reveals a possible infection.	Holdings with a non-disease-free status where *M. bovis* is isolated.	Holdings with a non-disease-free status and without any current positive test but undergoing health measures.
Status transition	In the presence of a positive test or detection of suspicious lesions, the herd passes to T3S.	These herds pass to T2.1 when the presence of *M. bovis* is confirmed. If not, they are classified as T3.	All animals over 6 weeks of age are tested 42 days after the removal of all positive animals. If there are not any positive tests, two consecutive tests are carried out a minimum of 60 days apart. If results obtained in the last tests are both negative the herd status passes to be T2.	All the bovines over 6 weeks of age are tested after 6 months of achieving this status. If all animals test negative the herd acquires the T3 status.
Allowed Movements	None between holdings with the T3 status.	Only allowed to move animals to slaughter, under official control.	Only allowed to move animals to slaughter, under official control.	Only allowed to move animals to slaughter, under official control.

Source: DGAV (6)
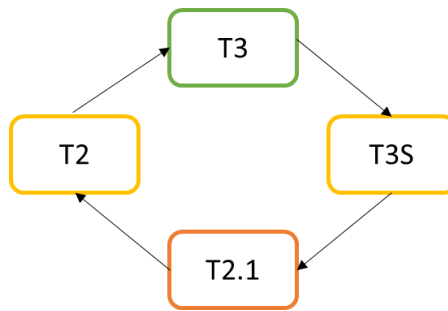
Figure 5: Herd status transition in Portugal. Source: DGAV (6).

The Portuguese eradication program has several benefits such as the elimination of a zoonotic agent, and the reduction of movement barriers between herds (in the disease-free herds - T3) (6). Another advantage of this programme is an increase of officially tuberculosis-free status areas, where fewer bTB diagnostic tests and analyses in slaughtered animals are done, thus encouraging livestock production (6). Despite its benefits, this programme is challenged by the presence of wildlife reservoirs, such as the wild boar and the deer (6). To overcome this obstacle there is big-game animals hunting's control: in all big hunting activities, one veterinarian conducts an initial examination of the carcasses, to ensure meat safety and collects bTB suspected lesions for laboratory diagnosis (6). Also, the correct and safe disposal of the by-products of hunting is encouraged (6). Furthermore, in areas where there is the risk of cattle contact with wild animals, cattle owners are advised to reinforce biosecurity measures to keep their animals separated from the wild ones by ensuring that feeding and watering places are not used by both types of animals (6).

Despite the existing control measures, control and eradication of bTB remain a challenge. One of the reasons is that these measures rely highly on testing, thus depending on the sensitivity and specificity of the diagnostic tests, detailed in the following section.

2.4.2 Diagnosis of bovine TB

The diagnostic methods are especially important for this disease as often there are no clinical signs (1). The only way to control transmission in cattle is through testing (1).

The development of tests started with the discovery of the "Tubercle bacillus" by Koch in 1882 which led to the development of tuberculin (1890) (30). In Denmark, the Koch's Old Tuberculin test was the official tool of the eradication program, during the early 1890s (30). However, a more stable substance with enhanced purity and potency was necessary (31). This was possible in the 1930s with the purification and the isolation of the tuberculin with antigenic proprieties, which produced the purified protein derivative tuberculin (PPD), part of the first trustworthy tuberculin skin test (31). From 1952 until

now, WHO has adopted PPD as the standard diagnosis technique, essential to implement the standard strategy against bTB: test and slaughter (30,31).

There are other testing techniques used as complementary assays or in research, such as IFN-ɣ test, serological assays, mycobacterial culture, and molecular typing (32). Nevertheless, tuberculin skin tests are still the most used diagnostic tests (30,31). The existing methods are summarized in Table 3.

In Portugal, the Single Intradermal Comparative Tuberculin Test (SICTT) is the mandatory test for bTB diagnosis (6). At standard interpretation, this skin test has an average specificity of 99.98% and a sensitivity within the range of 50-80% (6,33). Meanwhile, the IFN-ɣ test is a complementary assay to improve the sensitivity of the intradermal tuberculin test (1,6). IFN- ɣ is used when there are doubts in certain animals in non-disease-free explorations (1,6). When there are suspicious lesions in post-mortem routine examination at a slaughterhouse or a positive case from a herd that has not previously been infected (the herd is not classified as T2.1.), the isolation of *M. bovis* is necessary, through mycobacterial culture (6).

Worldwide, the strategies applied are testing with a tuberculin test and slaughtering (34). This tuberculin test has limited sensitivity, implying that on average 20-25% of bTB infected cattle can be missed (34). Therefore, it is important to complement this strategy with another practical one, such as vaccination.

Table 3: Diagnostic methods available for bovine tuberculosis (21,32,35–39).

Diagnostic methods	Definition	Advantages	Disadvantages
Single Intradermal Tuberculin Test (SITT)	Injection of the purified protein derivatives obtained from the heat-treated products of growth and lysis of *M. bovis.* Used in most European Union Member States.	- Higher sensitivity than SICTT. - Effective diagnostic test when applied at the herd level to identify *M. bovis* infection.	- Lack of sensitivity at the individual animal level. - Lower specificity than SICTT.
Single Intradermal Comparative Tuberculin Test (SICTT)	Injection of the purified protein derivatives obtained from the heat-treated products of growth and lysis of *M. bovis* and *M. avium.* Used in Great Britain, Ireland, and Portugal.	- Higher specificity than the SITT. - Effective diagnostic test when applied at the herd level to identify *M. bovis* infection.	- Lack of sensitivity at the individual animal level. - Lower sensitivity than SITT.
IFN-γ test	Based on the known production of IFN-γ when blood cells are incubated with *M. bovis*, following previous exposure to this pathogen. It detects a CMI[d] response to infection.	- Higher sensitivity than tuberculin skin tests.	- Lower specificity than tuberculin skin tests. - Expensive.
Serological assays	Immunological assay commonly used to measure antibodies, antigens in biological samples (e.g., ELISA).	-Useful as complementary tools to detect infected animals missed by cell-mediated response-based tests. -Blood sampling does not alter the immune status of the animal. -High specificity.	-Poor efficiency due to the late and irregular humoral immune response in bTB. -Less efficient to identify cattle in the early stages of infection when antibodies titres are low. -Low sensitivity.

Diagnostic methods	Definition	Advantages	Disadvantages
Mycobacterial culture	The gold standard for confirmatory post-mortem diagnosis of bTB.	-Agent identification.	-Difficulty in obtaining samples. -Slow growth of the agents and additional time for identification.
Typing methods	Identifies the mycobacterial species on a molecular basis. It is a valuable tool in epidemiological research and for the identification of potential sources of infection	-Rapid typing techniques, based on PCR (i.e., spoligotyping). -Important insights into the sources of infection, allowing the establishment of epidemiological links, necessary for the development of successful control and eradication strategies.	-Expensive.

SICTT- Single Intradermal Comparative Tuberculin Test, SITT- Single Intradermal Tuberculin Test, IFN-γ - Interferon-gamma, CMI- cell-mediated immune, ELISA- Enzyme-linked immunosorbent assay, bTB- Bovine Tuberculosis, PCR- Polymerase Chain Reaction amplification

2.4.3 Vaccination

Vaccines are used to prevent the development of clinical disease, reduce the spread of infection, and may prevent the infection itself (17). Vaccination is the most efficient tool for the prevention and control of infectious diseases, through high vaccination coverage for a prolonged period (40,41).

Vaccines are biological preparations that generate immunity by creating memory cells against a certain disease, through the administration of its agent (attenuated or inactivated) or a recognisable portion (toxins or surface protein subunits) (40,41). In this way, the vaccine will prepare the immune system to respond to future exposure to an antigen (40,41).

Regarding bTB, there is not any specific vaccine to be used in cattle (7,42–44). However, the vaccine used to prevent *M. tuberculosis* in humans is a live-attenuated strain of *M. bovis*, the BCG vaccine (7,42–44)*.*

BCG has some properties to be considered a desirable candidate vaccine for cattle, such as being: i) cheap to produce; ii) administered via different routes (oral, parenteral); iii) safe; iv) relatively stable; and v) derived from *M. bovis* (17). Previous research regarding cattle vaccination with BCG concluded that this vaccine could help in the prevention of tuberculosis in cattle (8,29,40,45). Furthermore, there is induction of a significant level of protection and reduction of the development of visible lesions (29). Vaccine efficacy against severe infection has been variable, ranging from 30.0% to 77.9% (29).

Until now the candidate vaccine against bTB is BCG alone or with a booster dose (8,10,40,45–48). The mechanism of protection of BCG is through the development of antigen-specific memory T cells, which are part of the CMI response (49). The booster dose cannot be BCG because it reduces the level of protection against bTB (8,10,40,45–48). Therefore, the booster dose should be DNA, protein and virus-vectored subunit vaccines which improve the protection against bTB, but only when combined with the BCG (8,10,40,45–48). The booster dose is important to increase and maintain the protection (8,10,40,45–48).

However, BCG does not protect all vaccinated animals and it reacts with the tuberculin skin test, which leads veterinarians to assume that a vaccinated animal could be an infected one (8,10,17,40,45–48). This vaccine interferes with the specificity of the tuberculin skin test, as BCG shares some antigens with the PPD, used in the tuberculin test (50). As tuberculin skin test is the cornerstone of surveillance in the eradication programmes, BCG is not currently used worldwide, and cattle vaccination with BCG is forbidden in the European Union (Council Directive 78/52/EEC13) (7).

Therefore, to use this vaccine a DIVA test, which is capable of distinguishing vaccinated from infected animals, would be required (9). The DIVA tests need to be a skin test, cheap, and sufficiently sensitive to be used for bTB control worldwide (9). Regarding the use of a DIVA test, in 2021, a field trial in England and Wales will start, where the BCG will be administered and the diagnostic test will be a DIVA skin test, DST-F (51,52). This test is a combination of three *M. bovis* antigens, three proteins: ESAT-6, CFP-10, and Rv3615c, that are either absent or are not immunogenic in the BCG vaccine (51,52). This combination has shown potential in detecting infected animals and differentiating them from the vaccinated ones (52). Trials are underway to determine whether this option would be safe and effective (51). If this trial is successful, the BCG and DST-F can be available in the field in 2025 (51).

The OIE and the European Union foresee that if a DIVA test is available the use of BCG could be approved, which would be a supplementary control bTB measure (50,51). When considering low- and medium-income countries, in many cases the test and slaughter strategy is not affordable or acceptable, turning vaccination a key factor to tackle this disease (45). The presence of a vaccine would prevent transmission among cattle through direct and indirect protection (42). Moreover, vaccination in contact areas of cattle with wild animals would be important to control spill over from wildlife (42).

2.5 Mathematical Models and Bovine Tuberculosis

Mathematical models are used to describe reality in a simple way (53). In the epidemiology of infectious diseases, mathematical models are commonly used to study infectious diseases transmission dynamics, including the study of how these diseases spread in the real world and what affects their dynamics (53,54). These models are particularly useful in guiding difficult policy decisions when several control strategies are being considered, as it is possible to simulate what would happen under various scenarios (53). For instance, these models permit to estimate the impact of a vaccine in a certain population without implementing it (53).

There are different types of mathematical models depending on the type of variables, population and timeline that will be used. For this reason, models can be dynamic or static, deterministic, or stochastic and individual or population-based models (55). When we are considering the time, a dynamic model accounts for time-dependent changes in the state of the system, while a static model calculates the system in equilibrium (55). In deterministic models, the rate of change of the state variables are described according to parameters which represent a population average. (55). This type of model represents the average behaviour and is used when the subgroups of the population are large (55).

On the other hand, in stochastic models, the state variables are described according to a probability distribution and they are used when the population is small (55). Finally, population-based models track groups over time, they do not explicitly incorporate individual-level heterogeneity and can be either stochastic or deterministic (55). The individual-based models track individuals over time, incorporate heterogeneity, and they can only be stochastic (55).

The combination of the prediction power of the mathematical models with the possibility of introducing the vaccine with a DIVA test would be relevant to know whether the vaccine would support bTB eradication in Portugal.

To the best of my knowledge, there are few articles related to the use of mathematical models to predict the impact of BCG vaccination to control bTB. These studies have investigated some aspects related to BCG vaccination: the range of DIVA test characteristics necessary to see a protective herd level benefit of vaccination, the sample sizes for field trials of cattle vaccination with BCG, and lastly a meta-analysis of the BCG effect on the burden of disease on cattle (50,56,57). Only one estimated the possible bTB eradication when the BCG is implemented (57). The scenarios analysis concluded that the low to moderate (<15%) prevalence settings could reach the bTB-free level if BCG were used in the next 10 years period (57). These studies suggest that the vaccination benefits depend on the sensitivity and specificity of the DIVA test. Moreover, in order to make BCG vaccination more attractive to farmers, the frequency and duration of the restrictions applied to farms need to be altered (50,56). Further studies are required to understand the impact of vaccination on disease transmission.

The use of mathematical models in Portuguese cattle has great potential to understand bTB dynamics when a vaccine is implemented in cattle.

2.6   Objective

In this master project, the main topic is bTB in mainland Portugal and its eradication through vaccination. The current eradication program has the objective of eradicating this disease from Portugal until 2025. For this reason, if a vaccine could reach it sooner it would be certainly advantageous for public health and the economy. Hence, this study main objective was to estimate the impact of a vaccine implementation, specifically the BCG vaccine, in the local eradication of bTB in mainland Portugal.

Considering this main objective, the specific objectives were to:

1. Build a dynamic, deterministic, and population-based mathematical model which represents bTB transmission within cattle with the already implemented strategies (test and slaughter) and with BCG vaccination;

2. Parameterize the model to the Portuguese reality;
3. Simulate scenarios of vaccine implementation according to various coverage levels.

3 Methods

3.1 Mathematical Modelling of Bovine Tuberculosis

To model the bTB situation in Portugal, it is necessary to understand the basic concepts of the mathematical modelling nomenclature, assumptions, and way of reasoning. In this section, mathematical modelling of infectious diseases is briefly reviewed. This information provides the foundation of the framework developed for bTB modelling.

3.1.1 Basic concepts in a mathematical model

Epidemiological modelling of infectious diseases is typically based on compartmental models, in which the host population is divided into compartments based on infection status (53).

The simplest model to represent the dynamics of an infectious disease is the SIR model, in which animals/people are divided into three compartments: the susceptible (S) to contract the disease, the infectious (I), corresponding to individuals who have had contact with the pathogen and can transmit the disease, and the recovered (R), who have long-lasting immunity. The total number of individuals in the model are presented as N (53). These compartments can be represented through a flow chart (Figure 6).
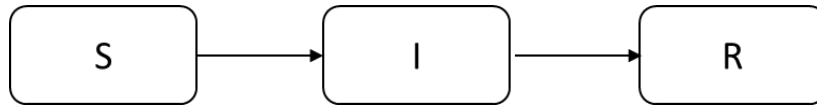
Figure 6: SIR model, which represents the disease transmission between susceptible (S), infectious (I), and recovered (R), without rates (56).

An infectious disease model establishes how the dynamic between compartments is behaving over time. The transition from S to I represents the origin of new infected animals/people and is given by the rate λ, the force of infection (53). The force of infection is non-constant and it is influenced by the prevalence of infectious (I/N), population contact structure (c), and the probability of transmission (p_t) (53). It can then be expressed as $\lambda = p_t * c * I / N$ (53). However, there is a simpler expression with the transmission coefficient, β, where $\beta = p_t * c$ (53). Hence, the force of infection equation (E1) is constituted with the β, the number of infectious animals/people (I) and the total number of animals/people (N). (53) The equation is the following (53):

$$\lambda = \beta * \frac{I}{N} \qquad\qquad (E1)$$

The transition from I to R is given by the recovery rate, γ, in which $1/\gamma$ represents the average infectious period (53). Besides the transitions between these compartments, it is also important to consider other ways of entering and leaving each compartment, which can be demographic events such as births (rate b), non-disease related deaths (rate μ), and disease-related deaths (rate α) (53). All the rates are summarized in Figure 7.
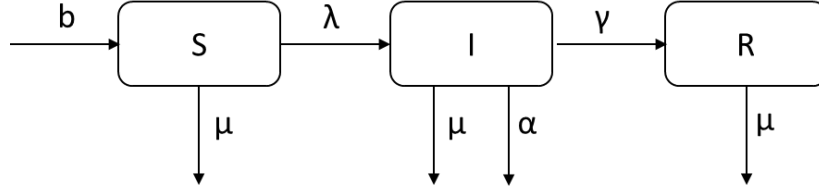
Figure 7: SIR model, with rates applied in susceptible (S), infectious (I), and recovered (R) individuals (53).

After establishing the flow diagram between the different compartments and rates of transmission, the next step is to translate this diagram into a set of ordinary differential equations. These ordinary differential equations depend on only a single variable, and they contain derivatives that translate the rate at each of the compartments change (58). The derivatives are the rate of change of a function concerning a variable, in this case, the time (59). Those rates are multiplied by the number of individuals in the group that they refer to (53). Such equations for the SIR model are presented below (E2 to E4) (53).

$$\frac{dS}{dt} = b * N - \mu * S - \lambda * S \tag{E2}$$

$$\frac{dI}{dt} = \lambda * S - (\mu + \alpha) * I - \gamma * I \tag{E3}$$

$$\frac{dR}{dt} = \gamma * I - \mu * R \tag{E4}$$

Another important concept for infectious disease modelling is R_0, which represents the average number of secondary cases occurring from a primary case in an entirely susceptible population (53). The R_0 for the SIR model can be calculated with the coefficient, β, multiplied by the average infectious period given by $1/\gamma$, which is impacted by all rates leaving the I compartment (α, μ), as it is shown in the following equation (E5) (53):

$$R_0 = \frac{\beta}{\gamma + \alpha + \mu} \tag{E5}$$

The R_0 measures the maximum reproductive potential of an infectious disease of a specific population (53). The disease will spread in the population when $R_0 > 1$ (53).

When $R_0 < 1$ the disease will get extinct in time (53). At $R_0 = 1$, the disease is neither growing nor diminishing (53):

The SIR model can include more compartments allowing the creation of models closer to reality (53). One of the possible changes is to add a latent period, which corresponds to the lag of time between acquiring the infection and being able to transmit it (53). This group is called Exposed (E), and the model obtained is the SEIR (53).

3.1.2 Modelling Bovine Tuberculosis

When modelling a specific disease, it is essential to capture the disease dynamics. Bovine tuberculosis has a long period of latency, which needs to be translated into the model. Furthermore, when there is a positive test, the animal is slaughtered. Thus, for the bTB model, the exposed compartment was added and the recovered compartment was not considered (53,60).

Therefore, a simple representation of bTB is a SEI model, where susceptible bovines change to the exposed compartment at a rate λ, the force of infection (28,53). The transition from the exposed compartment to the infectious is given by the rate δ, where $\frac{1}{\delta}$ represents the average duration of the latent period (53). Also, demographic events are important to define a model and they are implemented in this model as previously explained, except for the α which represents the culling rate. The culling rate is the rate at which the positive tested animals are slaughter (test and slaughter strategy). Furthermore, there is an extra rate that represents the rate of infection due to cattle contact with wildlife (w) (53,54). The following flow diagram frames this disease dynamics

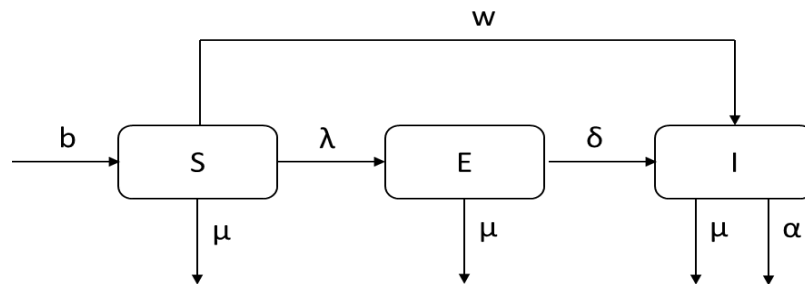*Figure 8: SEI model, including susceptible (S), exposed (E), and infectious (I) (53).*

in cattle (Figure 8) (53).

The differential equations (E6 to E8) that translate the rate at which each of the compartment's changes is presented below (53).

$$\frac{dS}{dt} = b * N - \mu * S - \lambda * S - w * S \qquad \text{(E6)}$$

$$\frac{dE}{dt} = \lambda * S - \mu * E - \delta * E \qquad \text{(E7)}$$

$$\frac{dI}{dt} = \delta * E - (\mu + \alpha) * I + w * S \qquad \text{(E8)}$$

To model bTB and BCG vaccination, a more complex framework is required. As previously mentioned, a bovine can get infected by *M. bovis* entering a latent period, where they cannot transmit the bacterium and are difficult to detect (check section 2.2.2) (1,11,15). Furthermore, to include vaccination in a model it is necessary to know how the vaccine will operate in cattle. It is known that BCG has a ranging efficacy, against developing tuberculous lesions and culture positive for *M. bovis* in cattle, between 30.0% to 77.9% (29). Therefore, BCG is considered a leaky vaccine, meaning this is a vaccine that reduces the infectiousness of vaccinated individuals but does not eliminate the risk of infection (56). Hence, even if all cattle are vaccinated some animals do not get fully protected (29,56). Yet, when these not-fully protected animals are compared to the non-vaccinated animals, the severity of pathology and dissemination of *M. bovis* is significantly lower (29,56).

To add the vaccination to the model it was essential to have three more compartments:

- Susceptible vaccinated (S_v);
- Exposed vaccinated (E_v);
- Infectious vaccinated (I_v).

In these compartments, the transmission rate is lower because the vaccine has some impact on the disease's transmission (29,56). Therefore, some animals can get infectious at a lower rate (29,56). Also, waning immunity was not considered because the animals are either considered to get revaccinated or culled. This model has a constant population and for this reason, the birth and the death rate are the same and there is a culling rate for I and I_v which is compensated by this rate entering in the S compartment again at the same rate. The S compartment represents susceptible animals, the E compartment represents exposed animals and the I compartment represents infectious animals. In both parts, there are the demographic rates, such as the birth rate (b), the natural death rate (μ), the wildlife infection rate (w) and the culling rate (α). Also, there are the transition rates between compartments, such as the force of infection (λ) and the latent rate (δ).

This model needed some adaptations due to the vaccine's interference with the disease transmission and as BCG is a leaky vaccine this interference only reduces, but do not eliminate, the risk of infection of vaccinates (56). In the force of infection, λ (E9) the infection by vaccinated infectious individuals was included, β∗IvN, multiplied by ci

(deemed to be equal to cs presented in E10), which is a term to reduces the infectivity. (50,61,62).

$$\lambda = \beta * \frac{I}{N} + ci * \beta * \frac{Iv}{N} \tag{E9}$$

$$vaccine\ efficacy = 1 - cs \tag{E10}$$

Furthermore, vaccination coverage (vc) is added to consider the proportion of S and E vaccinated (non-infectious compartments). This parameter influences the disease progression because vaccinated animals are less susceptible to be infected. The vaccinated compartments multiply the force of infection by a factor, cs, which represents the vaccine effect on susceptibility (50,61). In other words, this means that vaccination reduces the risk of infection by a factor cs (E10), which means that the vaccine impacts the infectivity and the susceptibility in the same way.

Besides, the transition between parts is made through the presence of vaccination coverage (vc) which happens between the S and S_v, and between E and E_v. Lastly, it is considered new-born vaccination, therefore the birth rate is multiplied by vc when considering the S_v compartment, whereas the birth rate is multiplied by 1-vc when considering the S compartment.

The following flow diagram (Figure 9) has three compartments representing the non-vaccinated population (SEI) and the other three compartments representing the vaccinated population ($S_vE_vI_v$).
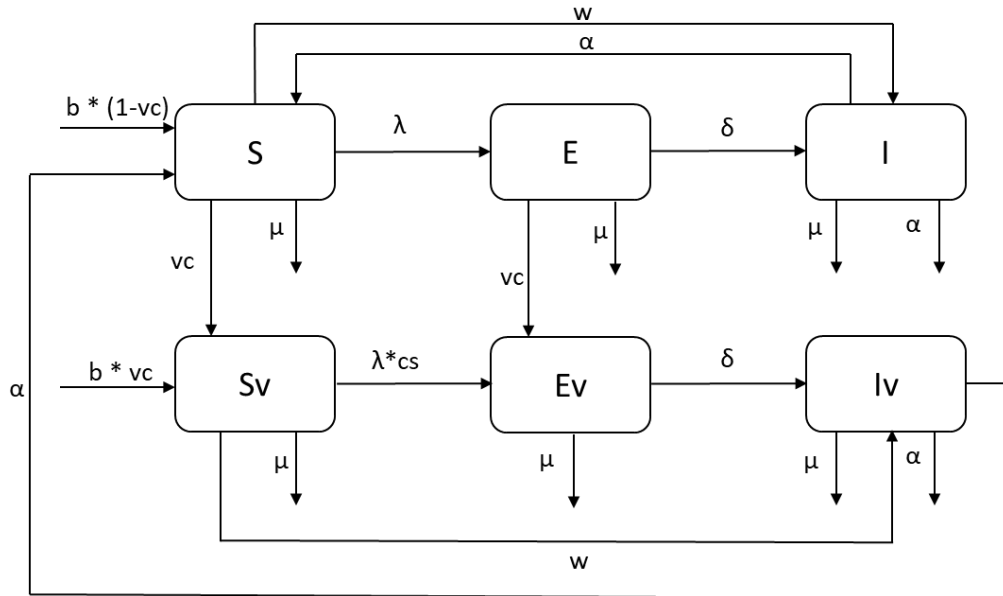

Figure 9: Flow diagram of bovine tuberculosis transmission among cattle including test and slaughter (α) strategy and vaccination. The model includes two parts: the compartments that represent the non- vaccinated animals (SEI) and the compartments that represent the vaccinated animals ($S_vE_vI_v$).

With all these changes the equations for the BCG vaccination at birth and continuous vaccination against the bTB model are presented below (E11 to E16).

$$\frac{dS}{dt} = \left(b * N * (1 - vc)\right) - \mu * S - \lambda * S - w * S - vc * S + \alpha * (I + Iv) \quad \text{(E11)}$$

$$\frac{dE}{dt} = \lambda * S - \mu * E - \delta * E - vc * E \quad \text{(E12)}$$

$$\frac{dI}{dt} = \delta * E - (\mu + \alpha) * I + w * S \quad \text{(E13)}$$

$$\frac{dSv}{dt} = b * N * vc + vc * S - \mu * Sv - \lambda * cs * Sv - w * Sv \quad \text{(E14)}$$

$$\frac{dEv}{dt} = vc * E + \lambda * cs * Sv - \mu * Ev - \delta * Ev \quad \text{(E15)}$$

$$\frac{dIv}{dt} = \delta * Ev - (\mu + \alpha) * Iv + w * Sv \quad \text{(E16)}$$

Additionally, R_0 needed to be calculated. However, the standard R_0 equation could not be used because the bTB has different characteristics, such as the existence of a latent period, no native immunity, and no possible recovery (all infected animals are slaughtered) (53,60). Also, the addition of the BCG vaccine which adds more complexity to the disease transmission dynamics needs to be taken into consideration. For this reason, the R_0 needs to be calculated with the next generation matrix (63,64).

3.1.2.1 Basic reproduction number deduction

R_0 is a threshold value that indicates whether the disease will invade a susceptible population or not (63,64). To deduct R_0 with the next generation matrix method it is necessary to identify the equations that describe the production of new infections and changes in state among infected individuals (63,64). As it is considered that an infectious agent is introduced into a fully susceptible population, it is assumed that the change in the susceptible population is negligible during the initial spread, which is called linearization (63,64). In this project, the infected states are the compartments E, I, E_v and I_v. Thus, the linearization of equations S+ S_v=N, which are represented in the following equations (E17 to E20):

$$\frac{dE}{dt} = (\beta I/N + ci\beta Iv/N) * S - \mu * E - \delta * E \quad \text{(E17)}$$

$$\frac{dI}{dt} = \delta * E - (\mu + \alpha) * I \quad \text{(E18)}$$

$$\frac{dEv}{dt} = (\beta I/NSv + ci\beta Iv/N) * Sv * cs - \mu * Ev - \delta * Ev \quad \text{(E19)}$$

$$\frac{dIv}{dt} = \delta * Ev - (\mu + \alpha) * Iv \quad \text{(E20)}$$

The equations in the infected subsystem are described by a matrix, which is called the Jacobin matrix when derived by linearization of the original nonlinear equations system (63,64). Firstly, the matrix is decomposed into two matrices, *F+V*, where the *F* is the transmission part, describing the new infections, and *V* is the transition part, describing the state changes (63,64). After defining these two matrices is calculated the dominant eigenvalue[1] of the matrix *FV⁻¹*, which is the R_0 (63,64). The *F* (E21) and *V* (E22) are presented below.

$$F=\begin{bmatrix} 0 & \beta/NS & 0 & ci\beta/NS \\ 0 & 0 & 0 & 0 \\ 0 & \beta cs/NSv & 0 & csci\beta/NS \\ 0 & 0 & 0 & 0 \end{bmatrix} \tag{E21}$$

$$V=\begin{bmatrix} \mu+\delta & 0 & 0 & 0 \\ -\delta & \mu+\alpha & 0 & 0 \\ 0 & 0 & \mu+\delta & 0 \\ 0 & 0 & -\delta & \mu+\alpha \end{bmatrix} \tag{E22}$$

The next generation matrix is given by *FV⁻¹*. *V⁻¹* and *FV⁻¹* obtained are presented in matrices (E23) and (E24). Based on the latter it is possible to find the analytical solution for the R_0: it is the highest eigenvalue of *FV⁻¹* (9.4). The R_0 obtained using this approach is presented in equation (E25).

$$V^{-1}=\begin{bmatrix} \frac{1}{\mu+\delta} & 0 & 0 & 0 \\ \frac{\delta}{(\mu+\delta)(\mu+\alpha)} & \frac{1}{\mu+\alpha} & 0 & 0 \\ 0 & 0 & \frac{1}{\mu+\delta} & 0 \\ 0 & 0 & \frac{-\delta}{(\mu+\alpha)(\mu+\delta)} & \frac{1}{\mu+\alpha} \end{bmatrix} \tag{E23}$$

$$FV^{-1}=\begin{bmatrix} 0 & \frac{\beta*\delta}{NS(\mu+\alpha)(\mu+\delta)} & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & csci\frac{-\beta*\delta}{NS(\mu+\alpha)(\mu+\delta)} & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \tag{E24}$$

$$R_0 = \delta(\beta + ci*\beta)(\mu+\alpha)(\mu+\delta+vc) \tag{E25}$$

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[1] It is a scalar associated with a given linear transformation of a vector space and having the property that there is some nonzero (74).

3.1.1   Data source

The available dataset was provided by DGAV through the Programme for the eradication of bovine tuberculosis in Portugal (variables information in section 9.1). This data was obtained by the routine testing of herds done by veterinarians, which fill in a DGAV platform with the test result with all the specificities (animal number, herd, place, etc). The data that was accessed from 2010 to 2019 and it had the objective of setting parameters that represented bTB in cattle in mainland Portugal (except Algarve) (6). The most important variables in this dataset were the positive cases, the total number of animals, and the total number of tests by year. These were used to parameterize the model according to the Portuguese reality (see below).

3.1.2   Parameterization

Values to replace the parameters are required to solve the differential equations proposed to study the transmission dynamics of bTB in Portugal. Parameters were extracted from the literature and the available data (check section 3.1.1) and are summarised in Table 4.

This model had a constant population which meant that the birth and mortality rates are the same. Rates were obtained from a previous publication, for which the bovines were considered to live until five years of age (65).

The model was implemented starting in 2010. Thus, the initial number of animals in compartment I was calculated by multiplying the total number of animals by the disease animal prevalence in 2010. Considering that the total number of bovines in mainland Portugal (expect Algarve) in 2010 was 1224000 and the bTB animal prevalence in the same year was 0.3%, it was possible to estimate that the initial number of infectious animals were 3672 (27,66). The number of exposed animals was assumed to be (1000) and the number of susceptible animals was 1219328, corresponding to the difference between the total number of animals and the number of infectious with exposed animals (27,66). The culling rate was calculated from the data, as the mean from the yearly rates from 2010 to 2019, obtained by dividing the number of tests performed by the total number of existing animals (the yearly culling rate values in section9.2). The value obtained was 77%. Also, the culling rate will vary between that value and 1 (0.77, 0.90, 1), to observe the impact of different culling rates. With this parameter, it is possible to estimate the impact of the already implemented strategy.

The transmission coefficient (β, 5.2) and the latent period (δ, 3.65) were abstracted from the literature, considering a previous study conducted in Spain, as this was deemed to be close to the Portuguese reality (67). Since prevalence in Spain in cattle herds ranges

from 2% to 5% and in Portugal it ranges from 0.2% to 0.5%, I decreased the transmission coefficient to reflect the Portuguese reality by changing the β and w until the prevalence was closer to the mean Portuguese prevalence (68). Also, as I was not considering herds but instead the cattle population, which had an animal prevalence mean from 2010 to 2019 of 0.1%, the β changed to 0.01 (26,27). The δ was kept the same as the study abovementioned as it corresponds to a disease characteristic.

Table 4:Description of model parameters and their values, and references. Units are years^{-1} or number.

Parameter description	Parameter symbol	Value (Number or years^{-1})	Reference
Initial number of susceptible cattle	S	1219328	(66)
Initial number of exposed cattle	E	1000	-
Initial number of infected cattle	I	3672	(27)
Transmission coefficient	β	0.01	Adapted from (67)
Rate at which infected cattle become infectious	δ	3.65	(67)
Vaccine effect on susceptibility	c_s	0.39	(50)
Vaccine effect in reducing infectivity	c_i	0.39	(50)
Mortality rate	μ	1/5	(65)
Culling rate	α	0.77, 0.9 and 1	Data source
Birth rate	b	1/5	(65)
Vaccination coverage	vc	0, 0.5, 0.7, 0.9 and 1	This study
Wildlife infection rate	w	0.0009	Adapted from (25)

The vaccine effect on susceptibility (cs) and infectivity (ci) were obtained from a previous study estimating the BCG effect on reducing the risk of vaccinated animals acquiring infection (1- BCG efficacy), as 0.39 (50).

As this study intended to explore the impact of vaccination as a control measure, the model was implemented under several scenarios, with vaccination coverage varying between zero and one (0, 0.5, 0.7, 0.9, 1).

Finally, the wildlife infection rate was obtained from a previous study assessing the role of wildlife as the source of infection in Spain, which estimated this rate as 0.131 yr^{-1} (25). This parameter had to be adapted through different simulations in combination with the β until it represented the Portuguese prevalence, which turned w to 0.0009 yr^{-1}. Therefore, with the adapted β and w, the prevalence in 2010 corresponded to 0.1%, which corresponds to the real mean animal prevalence (26,27).

3.1.3   Model Implementation

The set of equations proposed for the bTB and BCG implementation model combined with the parameters (Table 4) was used to estimate the impact of the vaccination as a complement to the test and slaughter strategy. The obtained results were the R_0 (using the formula presented in equation 25), the number of infectious animals in 2025, bTB animal prevalence in 2025 (%) and reduction in the infection (%) when compared with the current culling rate without vaccination.

All the results had a 10% variation in the culling rate to give a broader interval to analyse. Additionally, the culling rate ranged from 0.77 to 1 (0.77, 0.9, and 1) and the vaccination coverage ranged from 0 to 1 (0, 0.5, 0.7, 0.9, 1) to study the impact of different culling and vaccination rates in the bTB infection. All these variations in the model have the objective to test the robustness of the model.

The model was implemented using R software with the packages *deSolve* and *ggplot2* (69–71) and the code used is in section 9.3. The *deSolve* package solves a system of first-order ordinary differential equations (69). These equations contain one or more functions of one independent variable and the derivatives of those functions (69). Therefore, it was used for the numerical treatment of the systems of differential equations (69). The next step after solving the equations was the plotting, which was conducted using *ggplot2* (70).

4 Results

This section presents the graphic and numeric solutions of the proposed model for bTB with the BCG vaccine, which was used to study the role of control measures (test and slaughter and vaccination) in controlling the bTB transmission in Portugal. They are depicted graphically from Figure 10 to Figure 12; values for R_0, the number of infected animals (I and Iv compartments), the prevalence of bTB (infected animals' proportion) and the reduction in the infection are presented in Table 5. The difference between the figures and the table is the possibility to observe the variation of the vaccinated compartments in the figures, which is not possible in the table.

The impact of the vaccination to complement the test and slaughter strategy can be analysed through Figure 10, Figure 11, Figure 12 and Table 5. The three figures represent the impact of the vaccination (vc = 0, 0.5, 0.7, 0.9 and 1) with different culling rates (0.77, 0.9, 1). These figures indicate that the implementation of the vaccine would lead to a higher number of animals in all the vaccinated compartments, and this number increases with the increase of the vaccination coverage. As it can be observed in Table 5, the vaccination would have no impact on the reduction of bTB infection, because the number of infectious animals is the sum of the vaccinated and non- vaccinated infectious animals due to the supposed positivity on the diagnostic test in both cases.

Regarding the increase of the culling rate, a slight decrease in the number of infectious animals is observed both in the figures and the table. Also, as the culling rate increased, there was a reduction of infection, which reached 18% (9%- 27%) ($\alpha=1$) and bTB prevalence decreased slightly.

Overall, the results for bTB in 2025, even with different culling and vaccination rates, are close to each other, the number of infectious animals is around 1000, the bTB prevalence does not present a great difference (Table 5). Considering the R_0 (Table 5), which is lower than 1 in every simulation, therefore the infection is getting extinct slowly in time. However, there is a slight difference with the vaccination, where the R_0 turns to 0.01, whereas without the vaccination it is close to 0.05.

Figure 10: Graphical solution of the model including vaccination assuming the current culling rate (α=0.77) and varying the vaccination coverage (0, 0.5, 0.7, 0.9, 1), in log scale. S- susceptible, E- exposed, I- infectious, Sv- vaccinated susceptible, Ev- vaccinated exposed, Iv-vaccinated infectious.
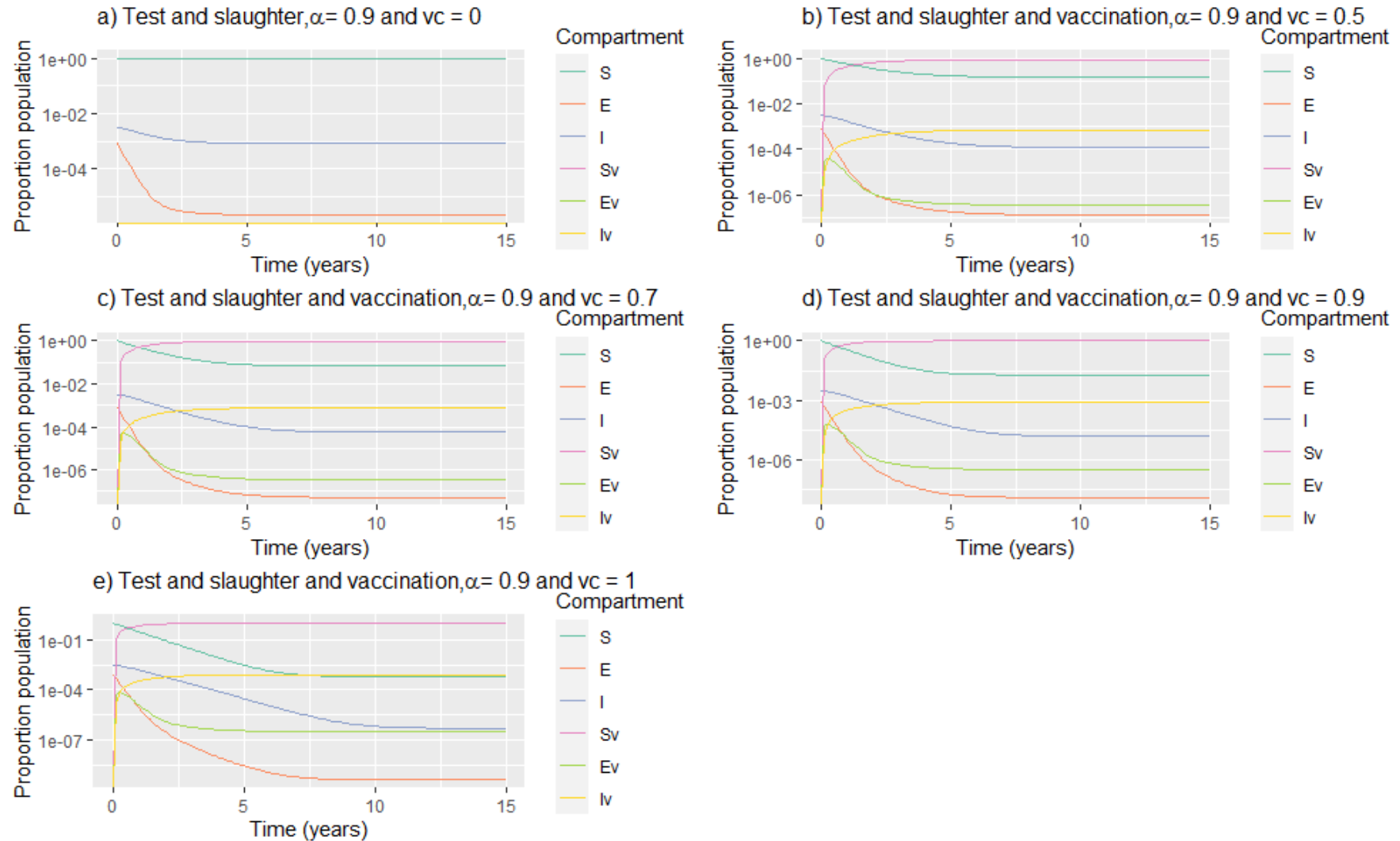
Figure 11 Graphical solution of the model including vaccination assuming a culling rate of 0.9 and varying the vaccination coverage (0, 0.5, 0.7, 0.9, 1), in log scale. S- susceptible, E- exposed, I- infectious, Sv- vaccinated susceptible, Ev- vaccinated exposed, Iv- vaccinated infectious.
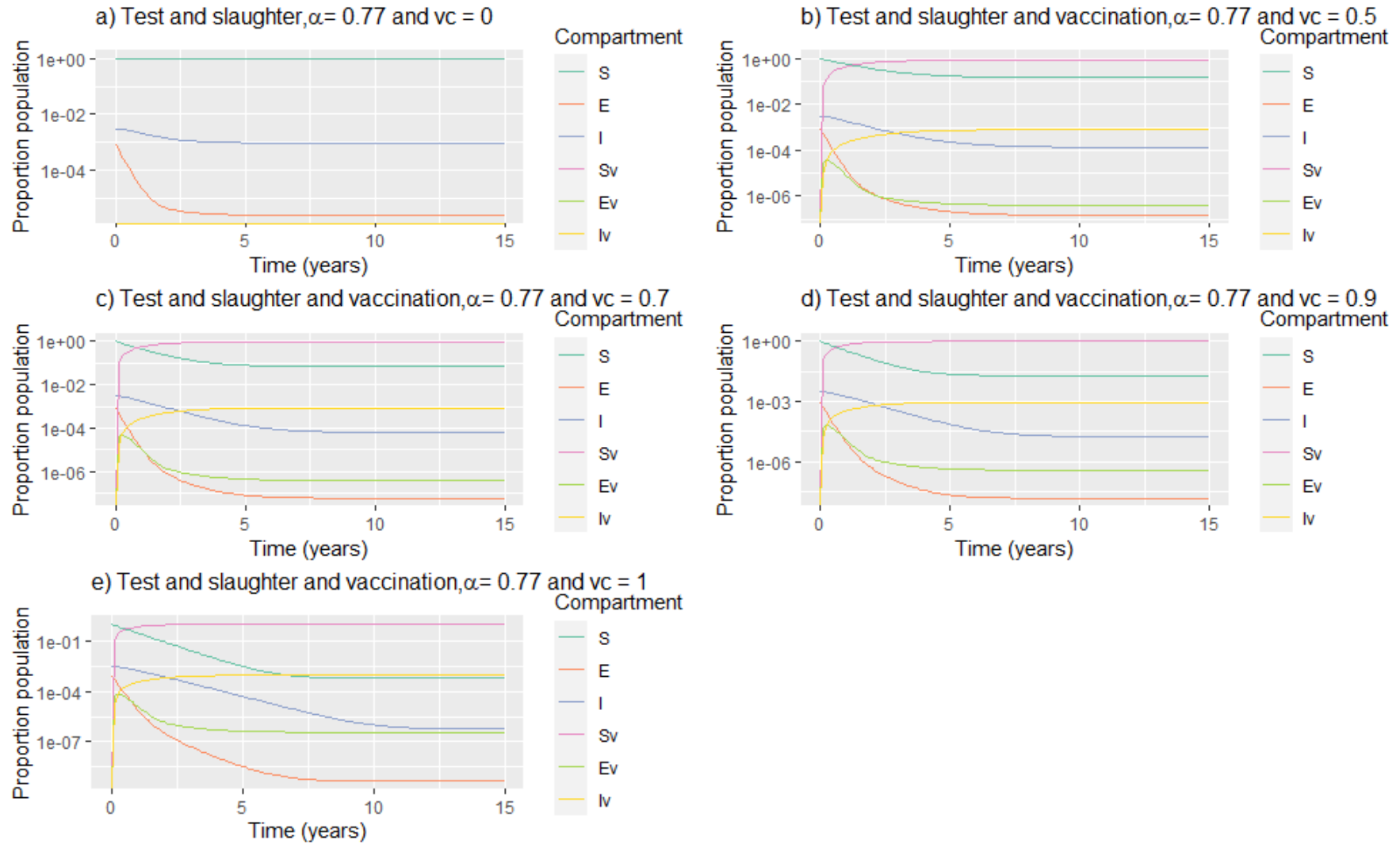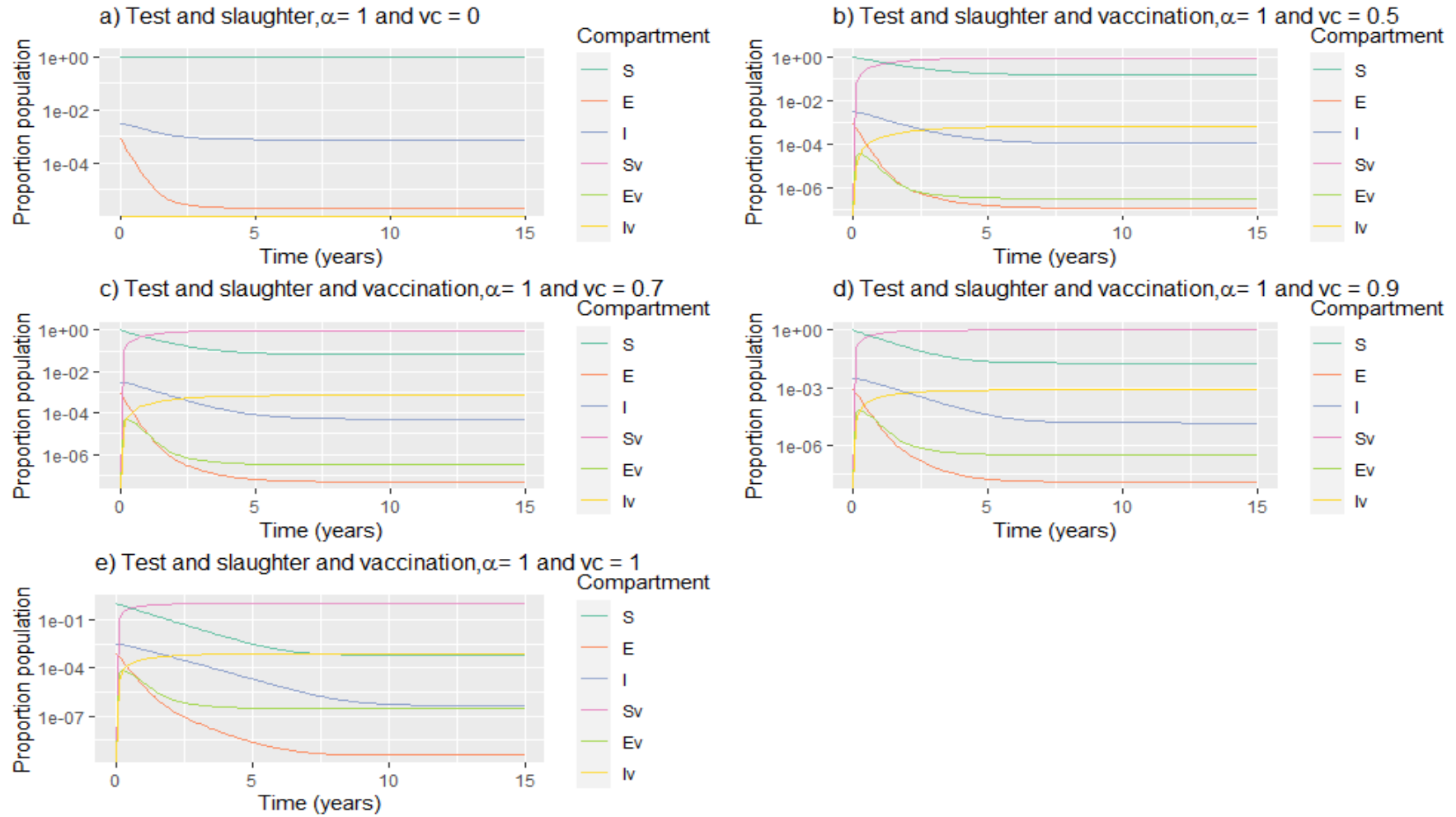
Figure 12: Graphical solution of the model including vaccination assuming a culling rate of 1 and varying the vaccination coverage (0, 0.5, 0.7, 0.9, 1), in log scale. S- susceptible, E- exposed, I- infectious, Sv- vaccinated susceptible, Ev- vaccinated exposed, Iv- vaccinated infectious.

Table 5: Numeric solution of the model in 2025.

Simulation \ Results	Varied Parameters			Results		
	Culling rate (10% variation)	Vaccination coverage	R_0	Number of infectious (I+ I_v) animals in 2025	bTB animal prevalence in 2025 (%)	Reduction in the infection (%)
TS (α=0.77, vc=0)	0.77 (0.69-0.85)	0	0.055 (0.056-0.054)	1100 (1200-1000)	0.090 (0.098- 0.082)	0 (0-9)
TS (α=0.9, vc=0)	0.9 (0.81-0.99)	0	0.053 (0.054-0.052)	1000 (1100- 900)	0.082 (0.090- 0.075)	9 (0-18)
TS (α=1, vc=0)	1 (0.9-1.1)	0	0.052 (0.053-0.051)	900 (1000- 800)	0.075 (0.083- 0.068)	18 (9- 27)
TS and V (α=0.77, vc=0.5)	0.77 (0.69-0.85)	0.5	0.016 (0.016-0.015)	1100 (1200-1000)	0.090 (0.098- 0.082)	0 (0-9)
TS and V (α=0.9, vc=0.5)	0.9 (0.81-0.99)	0.5	0.015 (0.016-0.015)	1000 (1100- 900)	0.082 (0.090- 0.075)	9 (0-18)
TS and V (α=1, vc=0.5)	1 (0.9-1.1)	0.5	0.015 (0.015-0.015)	900 (1000- 800)	0.075 (0.083- 0.068)	18 (9- 27)

Simulation \ Results	Varied Parameters			Results		
	Culling rate (10% variation)	Vaccination coverage	R_0	Number of infectious ($I+I_v$) animals in 2025	bTB animal prevalence in 2025 (%)	Reduction in the infection (%)
TS and V (α=0.77, vc=0.7)	0.77 (0.69-0.85)	0.7	0.012 (0.012-0.012)	1100 (1200-1000)	0.090 (0.098- 0.082)	0 (0-9)
TS and V (α=0.9, vc=0.7)	0.9 (0.81-0.99)	0.7	0.012 (0.012-0.012)	1000 (1100- 900)	0.082 (0.090- 0.075)	9 (0-18)
TS and V (α=1, vc=0.7)	1 (0.9-1.1)	0.7	0.012 (0.012-0.011)	900 (1000-800)	0.075 (0.083- 0.068)	18 (9- 27)
TS and V (α=0.77, vc=0.9)	0.77 (0.69-0.85)	0.9	0.010 (0.010-0.010)	1100 (1200-1000)	0.090 (0.098- 0.082)	0 (0-9)
TS and V (α=0.9, vc=0.9)	0.9 (0.81-0.99)	0.9	0.010 (0.010-0.010)	1000 (1100- 900)	0.082 (0.090- 0.075)	9 (0-18)
TS and V (α=1, vc=0.9)	1 (0.9-1.1)	0.9	0.010 (0.010-0.009)	900 (1000-800)	0.075 (0.083- 0.068)	18 (9- 27)

Simulation / Results	Varied Parameters			Results		
	Culling rate (10% variation)	Vaccination coverage	R_0	Number of infectious $(I + I_v)$ animals in 2025	bTB animal prevalence in 2025 (%)	Reduction in the infection (%)
TS and V (α=0.77, vc=1)	0.77 (0.69- 0.85)	1	0.009 (0.009- 0.009)	1100 (1200-1000)	0.090 (0.098- 0.082)	0 (0-9)
TS and V (α=0.9, vc=1)	0.9 (0.81-0.99)	1	0.009 (0.009- 0.009)	1000 (1100- 900)	0.082 (0.090- 0.075)	9 (0-18)
TS and V (α=1, vc=1)	1 (0.9-1.1)	1	0.009 (0.009- 0.009)	900 (1000- 800)	0.075 (0.083- 0.068)	18 (9- 27)

R_0-Basic reproduction number, bTB-Bovine tuberculosis, TS- Test and slaughter, V- BCG vaccination

5 Discussion

This work presents an initial attempt to assess the impact of cattle vaccination with the BCG vaccine, as a complement to the test and slaughter strategy. This project idea appeared because until now the test and slaughter strategy alone did not eradicate the bTB in Portugal and one of the major reasons is the presence of wildlife reservoirs. Thus, several possible scenarios of culling and vaccination rates were analysed using mathematical modelling. The model implemented had a 15-year timeline, starting in 2010, to assess what will happen until 2025, which is the objective set by the current eradication program to eradicate bTB in Portugal.

With the BCG implementation in the model, the number of vaccinated animals increased, turning the largest proportion of infectious animals into vaccinated ones. However, the overall number of infectious animals is kept the same when the culling rate is stable. When analysing which is the best vaccination coverage, as there is not any improvement in terms of the number of infectious animals, I could not establish which rate would give the best result because this model considers that vaccinated infectious animals are slaughtered. These results indicate that the vaccination needs to be complemented with the test and slaughter strategy because the BCG is a leaky vaccine with a wide range of vaccine efficacy (29). Nevertheless, in theory, there is a change of paradigm, where the severity of pathology and dissemination of *M. bovis* is significantly lower when the animals are vaccinated (42,56,72). Consequently, even if those animals are infectious, they have less probability of developing severe disease and transmitting to others (56). This is observed with the R_0, which showed a small impact of the vaccine in the disease transmission dynamics, because the R_0 was around 0.05 without vaccine and with the vaccine became around 0.01. This output was calculated through the next generation matrix because an explicit expression of it for the framework used in this project was not available in the literature.

Regarding, the culling rate, which was also analysed, the bTB prevalence decreased when this rate increased, showing the positive impact of the test and slaughter strategy. For this reason, the higher culling rate (1) would have some impact on the disease control. However, the test and slaughter strategy alone is not enough to eradicate bTB from mainland Portugal until 2025.

Overall, to answer the question analysed by this project: "Could a vaccine eradicate bovine tuberculosis in Portugal after 2025?", I would answer that BCG could not eradicate the bTB due to the lack of improvement in the number of infectious animals. Also, in other studies, the use of BCG against bTB on animals was analysed and the

results did not show the ability to eradicate this disease (50,56). This was concluded because there was not a substantial benefit of the BCG vaccination at the herd level when used as a supplement to test-and-slaughter strategy when comparing with the frequency and duration of restrictions applied to farms, which was not appealing for farmers that need to test and slaughter more to assess the vaccine, leading to a negative economic impact (50,56). These models estimate primary benefits from the DIVA test increased sensitivity rather than the vaccination itself (50,56). The results from this project are less specific than the other studies because I did not consider some specificities, such as the diagnostic test and restrictions applied to the farm. Yet the results are similar, which is reassuring.

Contrasting with this project, there is another study which is a systemic review and meta-analysis of the efficacy of BCG vaccination in cattle together with transmission dynamic model-based scenario which provided evidence for the implementation of BCG, particularly in low and medium-income countries, other high burden settings and in places with low bTB prevalence (<15%) (57). Even if BCG has a small protective effect, transmission models predicted that BCG limits the spread of the disease (57). Concluding that this vaccination may be enough to accelerate the control of bTB in Portugal (57). This difference of results between my project and this article could be because the model used was different and considered a compartment that represented infected, non-infectious, and non-detectable animals, and other which represented infected, non-infectious and detectable animals, due to the vaccination ability to decrease the infectivity and transmissibility. For this reason, fewer animals enter the infectious compartment and were able to transmit the disease.

This study also has some strengths. To the best of my knowledge, this mathematical model is the first one that tried to represent the bTB Portuguese reality and the control measures already applied and non-applied (vaccination). This model could be improved and used to analyse the impact of these control measures in Portugal or if you change some parameters in any other country. Moreover, with the power of this method, it was possible to analyse how changes in the test and slaughter and the vaccination would impact the bTB prevalence.

On the other hand, this model has some limitations. As it is a deterministic model, the force of infection is always set as the same, but with the implementation of control measures (a wider test and slaughter implementation and a vaccine) this disease would have a lower force of infection. In addition, the lack of data for Portugal, such as the transmission coefficient, and the real wildlife infection rate, limited the selection of parameters for this model. To address this, parameters from studies deemed to be closer

to the Portuguese reality were adopted. However, this strategy is still limited because it was necessary to try different values until the bTB prevalence reached the mean reality. Furthermore, by doing this the force of infection and wildlife infection rate could not be close to the reality (both rates are changed at the same time). Also, it would be interesting to apply a model which included the occult compartment, representing the animals with less infectivity due to the vaccine. Overall, this model lacks accuracy as it does not reproduce all the aspects that influence bTB, such as the herd size, real wildlife impact, the diagnostic test, cattle movement between holdings, and infection through the environment. These aspects are important to establish the routes of transmission of bTB. The presence of data related to the latter aspects would help to determine the long-term outcome of vaccination (56). However, the infection through the environment or wildlife is difficult to measure. Also, the lack of fieldwork makes this model hard to validate, which means that until some field trial related to BCG vaccination in cattle takes place in Portugal the real impact of vaccination cannot be measured.

To create a better model in the future, it should be a stochastic herd-level model representing the high-risk areas, because it should take into consideration the randomness of disease transmission, focus on specific herds instead of the entire country, specifically in the high-risk areas because the cases are concentrated in specific settings. Moreover, a stochastic model considers randomness, and it is recommended when a small number of infected animals or people are involved as when this happens transmission and spread can start due to chance. (53) This type of model was not considered in this work due to the lack of experience of the author on mathematical modelling.

Portugal has a low bTB prevalence and maybe the benefits would exist if this vaccination strategy was implemented only in high-risk areas, which normally have more wildlife contacts, rather than at the country-level as this model represented. The presence of significant wildlife reservoir hosts in Portugal, such as the wild boar, limits the progress of the bTB eradication program (42). As there is not a current control strategy to impede the spread from the wild animals and without it will be difficult to eradicate *M. bovis* in the contact areas of cattle with wildlife (42). For this reason, it is important to establish new strategies to control spill overs and some studies suggest that wild animals vaccination with BCG could reduce this risk (57,73). This deterministic model will be a starting point for the development of more complex and realistic models for bTB in Portugal or another country if the parameters are adapted. It could help to assess the impact of different control measures, such as the BCG vaccination, to later implement them in the reality.

6 Conclusion

This deterministic mathematical modelling of bTB in mainland Portugal included a vaccine besides the current test and slaughter strategy. The effect of different scenarios with different culling rates and vaccination coverages were analysed. The R_0 in all scenarios was below one which represented the absence of a bTB outbreak due to the low prevalence of this disease in Portugal.

The limited knowledge on the parameters which represented the Portuguese reality difficulted the creation of a more accurate model. Also, the dynamics of the model did not match completely the bTB reality in Portugal. However, this work built the foundation for improvement and highlighted some potentials and weaknesses of mathematical models.

Regardless of all the limitations, this study suggests that neither the implementation of BCG nor the current strategy alone would eradicate the disease until 2025.

7 Recommendations

Future research on bTB and vaccination in Portugal should explore stochastic models to consider the randomness of the disease transmission. Additionally, it should consider externalities such as the impact of wildlife contact, and animals' movements, the herd size, and diagnostic tests which have an impact on the disease dynamics.

Finally, the next mathematical models should focus on herds in high-risk areas, since Portugal has a low bTB prevalence and its cases are in its majority located in certain regions. This would help to better predict the reality. Furthermore, when a DIVA skin test is available if there is a good prediction with the model, field trials should take place to decide on the implementation of BCG.

This project shows the importance to set a new eradication objective and intensifying the control measures already applied, considering alternative options in particular for wildlife contact areas.

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9 Appendices

9.1 Bovine   tuberculosis   eradication   programme   database's   variable
       operationalisation plan

Table A: Variable operationalisation plan of the bTB eradication programme database.

Informatic name	Variable	Type	Values
Herd	Herd ID	Nominal	Alphanumerical
Year	Year	Numerical	2010 to 2019
Intervention_date	Testing date	date	date
Total_number_animals	Total number of bovines	Numerical	1 to 29775
Total_tests	Total number of tests	Numerical	1 to 2279
Negative	Number of negative results	Numerical	0 to 2279
Incomplete	Number of Incomplete results	Numerical	0 to 22
Doubtful	Number of doubtful results	Numerical	0 to 113
Positive	Number of positive results	Numerical	0 to 87

9.2 Yearly culling rate in Portugal in bovines.

Table B: Yearly culling rate in Portugal from 2010 to 2019.

Year	Culling rate (%)
2010	71
2011	75
2012	73
2013	74
2014	75
2015	74
2016	74
2017	84
2018	84
2019	84

Source: bTB Eradication program of Portugal.

9.3 Function created to solve the model in R studio.

```
# LOAD THE PACKAGES:

library(deSolve)

library(reshape2)

library(ggplot2)

# MODEL INPUTS:


initial_state_values <- c(S = 1224000- 4672,

                E = 1000,

                I = 3672,

                Sv = 0,

                Ev = 0,

                Iv = 0)


# Parameters


parameters <- c(beta = 0.01,     # the infection rate in units of years^-1

          delta = 0.01*365,     # the latency period in units of years^-1

          c_s = 0.39,      # the reduction in the force of infection

          # acting on those vaccinated

          c_i = 0.39,# the reduction in the infectivity of vaccinated infected bovines

          u = 1/5,#death rate in units of years^-1

          a = 1, #testing rate in units of years^-1 (changes)

          b = 1/5, #birth rate in units of years^-1

          vc = 1,  # vaccination rate (changes)

          w = 0.0009 ) #wildife infection rate in units of years^-1


# TIMESTEPS:
```

```r
# Sequence of timesteps to solve the model at

times <- seq(from = 0, to = 15, by =0.1)#from 0 to 15 years, daily intervals

# MODEL FUNCTION:


vaccine_model <- function(time, state, parameters) {


  with(as.list(c(state, parameters)), {


    # Defining lambda as a function of beta and E:

    N <- S + E + I + Sv + Ev + Iv

    lambda <- beta * I/N + c_i * beta * Iv/N

    # The differential equations

    dS <- -lambda * S - u * S - S * w - vc * S + (b * N * (1-vc))+ a * I + a * Iv

    dE <- lambda * S - delta * E - u * E - vc * E

    dI <- delta * E - a * I - u * I + S * w

    dSv <- -c_s * lambda * Sv - u * Sv + vc * S  + b * N * vc - Sv * w

    dEv <- c_s * lambda * Sv - delta * Ev - u * Ev + vc * E

    dIv <- delta * Ev - a * Iv - u * Iv + Sv * w


    return(list(c(dS, dE, dI, dSv, dEv, dIv)))
  })


}



# MODEL OUTPUT:

# Solving the differential equations using the ode integration algorithm
```

```r
output <- as.data.frame(ode(y = initial_state_values,

                times = times,

                func = vaccine_model,

                parms = parameters))


# PLOT THE OUTPUT

# turn output dataset into long format

output_long <- melt(as.data.frame(output), id = "time")


# Adding a column for the prevalence proportion to the long-format output

output_long$prevalence <- output_long$value/sum(initial_state_values)


# Plot the number in each compartment over time in log scale

ggplot(data = output_long,

    aes(x = time, y = prevalence, colour = variable, group = variable)) +

  geom_line() +

  xlab("Time (years)")+

  ylab("Proportion population") +

  labs(title = expression(paste("a) Test and slaughter,", alpha, "= 1 and vc = 1")),

    colour = "Compartment") +

  scale_colour_brewer(palette = "Set2") +

  theme(plot.title = element_text(vjust = 3)) +

scale_y_continuous(trans='log10')
```

9.4 Calculation of the eigenvalue.

$$|FV^{-1} - I\lambda| = 0$$

Here the λ is the scalar.

$$\begin{bmatrix} -\lambda & \dfrac{\beta*\delta}{NS(\mu+\alpha)(\mu+\delta)} & 0 & 0 \\ 0 & -\lambda & 0 & 0 \\ 0 & 0 & csci\,\dfrac{-\beta*\delta}{NS(\mu+\alpha)(\mu+\delta)} - \lambda & 0 \\ 0 & 0 & 0 & -\lambda \end{bmatrix} = 0$$