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Genetic selection of cattle for reduced bovine tuberculosis transmission

Duygu Madenci

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Declaration

I hereby declare that this thesis, submitted for the degree of Doctor of Philosophy at the University of Edinburgh, is entirely my own work and research, except where explicitly stated otherwise. It has not been submitted, in whole or in part, for any other degree or professional qualification at any institution.

Duygu Madenci

I dedicate this thesis to my mother Songül.
Thank you for believing in me even when I doubted myself; for teaching me resilience in the face of challenges; and for reminding me of the value of perseverance and humility.

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List of Abbreviations

APHA	Animal and Plant Health Agency
AHDB	Agriculture and Horticulture Development Board
bTB	Bovine tuberculosis
BCG	Bacille Calmette-Guérin
BCBC	British Cattle Breeders Club
BCS	Body condition score
BCMS	British Cattle Movement Service
BINSC	Binary Secondary Cases
DAMF	Department of Agriculture, Food and Marine
DD	Digital Dermatitis
DIVA	Differentiating Infected from Vaccinated Animals
DEFRA	Department of Environment, Food and Rural Affairs
DAERA	Department of Agriculture, Environment and Rural Affairs
EBVs	Estimated Breeding Values
EGENES	Edinburgh Genetic Evaluation Services
EEC	European Economic Community
EU	European union
ELISA	Enzyme linked immunosorbent assay
GLM	Generalized linear model
GLMMs	Generalized linear mixed models
HRA	High-Risk Areas
HPD	Highest Posterior Density
IFN-γ	Interferon gamma
ICBF	Irish Cattle Breeding Federation
LRT	Likelihood Ratio Test
LA	Lameness advantage
M. bovis	Mycobacterium bovis

MTBC	Mycobacterium tuberculosis complex
MIR	Mid-infrared
MCMC	Markov Chain Monte Carlo
NSC	Number of Secondary Cases
OTF	Officially Tuberculosis-Free
PMEs	Post-mortem examinations
PTAs	Predicted Transmitting Abilities
PPD	Purified protein derivative
£PLI	Profitable Lifetime Index
PCR	Polymerase Chain Reaction
RABDF	Royal Association of British Dairy Farmers
RMSE	Root Mean Square Error
SICCT	Single Intradermal Comparative Cervical Tuberculin
SIT	Single Intradermal Test
SIR	Susceptible-Infectious-Recovered
SNPs	Single Nucleotide Polymorphisms
SIRE	Susceptibility, Infectivity, Recoverability Estimator
SCC	Somatic cell count
TB	Tuberculosis
TST	Tuberculin skin test
USDA	United States Department of Agriculture
WHO	World Health Organisation
WOAH	World Organisation for Animal Health
ZIP	Zero-Inflated Poisson

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Abstract

Bovine tuberculosis (bTB) is a major cattle disease with significant economic impact on production in multiple countries. In the United Kingdom, bTB remains a critical challenge, especially in high incidence areas despite control programmes being in place. These programmes consist of regular skin testing and culling of test positive cattle, movement restrictions, wildlife control, and other biosecurity measures. Nevertheless, successful eradication of the disease has yet to be achieved. Previous studies have suggested that breeding cattle for enhanced bTB resistance can complement existing eradication efforts. However, breeding for increased resistance alone may not be sufficient to help achieve the national target to eradicate bTB in the next decade. Therefore, genetic selection for low bTB infectivity, in addition to high bTB resistance, has been proposed as a possible solution to accelerate this process. However, the genetics of bTB infectivity need to be investigated in order to demonstrate the feasibility of including infectivity into national breeding programmes.

The overall aim of this thesis is to investigate statistical evidence of genetic variation underlying bTB infectivity of cattle in Great Britain (GB) and assess the feasibility and implications of considering infectivity as an additional breeding goal.

The primary objective of this thesis is to define a bTB infectivity phenotype, which is crucial for understanding how infection spreads among cattle. Additionally, the thesis aims to examine various factors affecting bTB infectivity,

including environmental, management, and host genetic influences that might contribute to variation in disease transmission. Another key objective is to estimate genetic parameters related to bTB infectivity. Using these estimates, the thesis derives and assesses estimated breeding values (EBVs) for bTB infectivity. Finally, the thesis investigates the effect of selecting for reduced bTB infectivity.

Chapter 1 presents a literature review on bTB, its transmission and pathogenesis, diagnosis and current control strategies. Furthermore, the thesis aim, objectives and outline are presented.

Chapter 2 explores bTB data from Great Britain on both phenotypic and genetic levels and introduces the concept of the "index case approach" to define novel bTB infectivity phenotypes. Index case here refers to the first single positively tested animal in a herd that signals the start of a bTB breakdown. bTB infectivity is then defined as the number of secondary cases (NSC) attributed to the index case. Linear mixed models and generalized linear mixed models (GLMMs) are used to explore the effect of multiple factors and derive estimates of the genetic variance of bTB infectivity. The results produce the first estimates of genetic variation and heritability in the bTB infectivity. However, more advanced statistical models need to be explored to improve model fit and provide deeper insights into bTB infectivity and transmission dynamics.

In Chapter 3, Markov Chain Monte Carlo (MCMC) techniques are applied to fit GLMMs that can account for potential overdispersion and zero inflation issues in the data. Four different GLMMs (specifically Poisson, Zero-Inflated Poisson

(ZIP), Hurdle Poisson, and Geometric models) are employed to detect and estimate genetic variation in infectivity. Factors affecting bTB infectivity from Chapter 2 are included in these models. The results show that genetic variation in bTB infectivity exists and is estimable. Furthermore, sire estimated breeding values (EBVs) are derived for bTB infectivity. Based on the estimated posterior mean genetic variances obtained, sire selection leading to a reduction in infectivity by one genetic standard deviation would result in a 32 - 44% decrease in the expected NSC per index case.

Chapter 4 focuses on the implications of incorporating bTB infectivity into breeding programmes. The potential reduction in the number of secondary cases is calculated from hypothetical genetic gains achieved from selection based on sire EBVs from Chapter 3. Results show that, in order to achieve 10% reduction in number of secondary cases using the infectivity phenotype introduced in this thesis, strong genetic progress would be required. This may involve high selection intensity, accurate EBV evaluations, and consistent use of genetically superior animals for breeding. Furthermore, correlations of sire EBVs for bTB infectivity with bTB resistance and other economically important traits are examined, using the infectivity EBVs estimated in Chapter 3 and existing EBV estimates for the other traits. Results show no antagonistic correlations with the other traits, suggesting no potential adverse effects from selecting for reduced infectivity.

In conclusion, this thesis proposes and examines a proxy infectivity phenotype for bTB. Exploration of the GB data reveals that there is underlying genetic

variation in bTB infectivity, suggesting that the trait can be improved with genetic selection. Our results also suggest that implementing selection to decrease bTB infectivity is feasible and effective, and does not have any expected negative effect on other traits that are considered in the current breeding programme. Further studies are recommended to refine the infectivity phenotype for more accurate genetic evaluation and effective genetic selection for low bTB transmission.

Lay Summary

Bovine tuberculosis (bTB) is a serious disease affecting cattle worldwide, causing significant financial losses to farmers. In the UK, controlling bTB remains a major challenge despite ongoing programmes such as regular testing, culling infected cattle, and wildlife control. Current control programmes have their limitations, and full eradication of the disease has not yet been achieved.

Alongside other traditional disease control measures, an additional complementary strategy would be to breed cattle that are more resistant to bTB. However, focusing only on resistance may not be enough to eliminate the disease in the near future. An additional genetic strategy alongside breeding for resistance could be breeding cattle that are less likely to spread the infection to others, but more research is needed to understand how the genetics of cattle affect their infectivity, i.e., the ability to transmit bTB.

This thesis explores the genetics of bTB infectivity in dairy cattle. The research aims to provide evidence that there is genetic variability in bTB infectivity, which means some cattle are more likely to spread the disease than others. Using data from Great Britain, the study defines an approach to estimate infectivity based on how many secondary cases arise from the first identified infected animal, defined as the "index case." The research uses various statistical models to analyse this data, ultimately showing that it is possible to breed cattle that are less likely to spread bTB.

The results show that genetic variation in bTB infectivity does exist. They also suggest that selecting cattle with lower infectivity could reduce the spread of bTB by up to 44%. Additionally, this breeding strategy does not negatively impact other important traits. Overall, the thesis concludes that breeding for lower infectivity could complement current control measures and accelerate efforts to eradicate bTB in cattle without harming other cattle traits.

Chapter 1

Introduction

1.1 Bovine Tuberculosis

1.1.1 Aetiology

Bovine tuberculosis (bTB) is a chronic infectious disease that has historically been a significant problem in livestock industries worldwide due to its impact on animal welfare, agricultural productivity, and trade (Barnes et al., 2023). Despite extensive control and eradication efforts, bTB remains a persistent and challenging disease worldwide (Admassu et al., 2015).

Cattle are considered the primary reservoir host for *Mycobacterium bovis (M. bovis)*, the bacterium responsible for bTB. *M. bovis* is a member of the *Mycobacterium tuberculosis complex* (MTBC), which includes other species such as *M. tuberculosis, M. africanum, M. caprae, and M. microti* (Michel et al., 2010; Admassu et al., 2015). These species share high genetic relatedness (Smith et al., 2006) and are responsible for tuberculosis (TB) in humans (primarily caused by *Mycobacterium tuberculosis*), livestock and wildlife (primarily caused by *Mycobacterium bovis*), making MTBC a significant public and animal health concern worldwide (Thoen et al., 2006; Michel et al., 2010; Thoen et al., 2014).

The main causative agent of bTB, *M. bovis*, is a slow-growing, aerobic, gram-positive, acid-fast bacillus that requires oxygen to survive (Rito et al., 2023). It

has a generation time of up to 20 hours. Similar to other members of the MTBC, *M. bovis* primarily targets the respiratory system, forming granulomatous lesions, commonly known as tubercles, which are primarily found in the lungs and associated lymph nodes (Domingo et al., 2014).

1.1.2 Epidemiology, transmission and host range

The disease in cattle is primarily transmitted through the inhalation of aerosolized bacteria (Goodchild et al., 2015), although transmission can also occur through other routes, including direct contact and contaminated feed or water (Phillips et al., 2003). Direct contact occurs when infected cattle transmit the bacteria through nose-to-nose contact or shared equipment. Aerosol transmission is impactful when infected animals release bacteria into the air via respiratory secretions, which can then be inhaled by other cattle (Skuce et al., 2011; Pérez-Lago et al., 2014). Contaminated feed or water may serve as transmission routes if they are fouled with excretions from infected animals (Michel et al., 2007). Wildlife reservoirs also play a crucial role in the epidemiology of bTB, contributing to the persistence and spread of the disease among cattle and other wildlife (Byrne et al., 2024).

Infected cattle may exhibit symptoms such as coughing, weight loss, and swollen lymph nodes, although many animals remain asymptomatic, acting as silent carriers and facilitating the spread within herds (Ramos et al., 2015).

bTB is a zoonotic disease that can spread between animals and humans (Conteddu et al., 2024). When *M. bovis* causes human infections, it can lead to severe illness and, in rare cases, even death. This happens particularly in developing countries where efforts to control and eliminate diseases are often weak (Rahman et al., 2020). In these regions, lack of resources and limited access to healthcare exacerbate the impact of disease on human populations. According to the World Health Organization (WHO), in 2021, TB caused over 1.6 million human deaths globally. This was an increase compared to previous years, breaking a trend of declining deaths seen between 2005 and 2019. The increase in TB deaths highlights how the COVID-19 pandemic disrupted TB treatment and control, showing the urgent need for stronger global efforts to fight this disease (WHO, 2022). While the majority of these deaths are due to Mycobacterium tuberculosis, the primary human pathogen, a significant number are caused by zoonotic TB, particularly *Mycobacterium bovis* and increasingly Mycobacterium orygis in regions like the Indian subcontinent. Recent data suggest that at least ~12,000 of the annual human TB deaths are attributable to zoonotic transmission (Duffy et al., 2024). This reinforces the importance of controlling TB not only as a human health issue, but also as a broader One Health challenge with implications for animal and public health worldwide.

bTB presents public health risks, especially in regions where milk pasteurization is limited and human and animal interactions are close (O'Reilly & Daborn, 1995). Although rare in developed agricultural systems, zoonotic transmission can occur through direct contact with infected animals or consumption of

unpasteurized dairy products (Ayele et al., 2004). Efforts to control bTB in cattle are thus critical for livestock protection and contribute to the broader One Health approach, which recognises the close connections between human, animal, and environmental health. (WHO, 2005).

The transmission of bTB can also occur between cattle and wildlife species through direct contact or indirectly via contaminated environments, such as shared grazing areas or water sources. For example, European badgers (*Meles meles*) are known to be a significant reservoir of bTB, leading to ongoing transmission between wildlife and cattle (Broughan et al., 2016). This interspecies transmission further complicates control and eradication efforts.

Control and eradication programs have traditionally relied on a combination of test and slaughter strategies and stringent biosecurity measures. However, these efforts are often complicated by multiple factors, including the presence of wildlife reservoirs (e.g., badger and deer), as well as residual (hidden) infection within cattle herds. The latter is often due to limitations in current diagnostic tests and testing regimes, which can allow infected animals to remain undetected (Gormley & Corner, 2018; More, 2024). As a result, many countries continue to struggle with complete eradication, and growing evidence suggests that success depends not only on wildlife control but also on risk-based approaches such as regionalization and risk-based trading (More, 2024).

Across Europe, several countries including Denmark, Finland, Sweden, Germany, Austria, and the Netherlands, have successfully eradicated bTB (Wahlström et al., 2010; Foddai et al., 2015; More et al., 2017; Orrico et al.,

2022). In contrast, Ireland continues to face persistent infection, with both wildlife (particularly badgers) and cattle-related transmission playing significant roles in ongoing infection, despite decades of testing programs and badger vaccination trials (Good & Duignan, 2011; More, 2024). Spain also faces similar challenges, particularly in areas with high interaction between cattle and wildlife, such as wild boar *(Sus scrofa)* and red deer *(Cervus elaphus)* (Gortazar et al., 2011).

In the United Kingdom(UK), bTB remains endemic in certain areas, particularly southwest England and Wales. Both cattle-to-cattle transmission and the presence of badger populations contribute to the resilience of the disease in these regions, complicating eradication efforts (Delahay et al., 2007; Walsh et al., 2008; Skuce et al., 2011; Godfray et al., 2013; DEFRA, 2018).

Beyond Europe, the United States (U.S.) has largely controlled bTB in domestic herds through the National Tuberculosis Eradication Program (Naugle et al., 2014). However, wildlife reservoirs, particularly white-tailed deer (*Odocoileus virginianus*), continue to pose challenges. These wildlife populations can act as sources of infection for cattle, complicating eradication efforts (O'Brien et al., 2023). Canada has made significant progress in controlling bTB, though rare cases occur, often linked to wildlife reservoirs such as bison *(Bison bison)* and elk *(Cervus canadensis)* (Leighton, 2011; Fitzgerald & Kaneene, 2013).

1.2 Prevalence of bTB

The global prevalence of bTB varies significantly, with some countries having achieved Officially Tuberculosis-Free (OTF) status while others continue to face challenges in controlling the disease due to wildlife reservoirs, economic limitations, and inadequate veterinary infrastructure.

The European Economic Community (EEC) introduced the concept of OTF status in 1964, requiring countries to maintain a herd infection rate below 0.1% for six consecutive years to qualify (Gordejo & Vermeersch, 2006; More et al., 2017; Allen et al., 2018). As of 2017, 18 EU member states, including Denmark, Finland, Sweden, Germany, Austria, and the Netherlands, had achieved OTF status. For example, Denmark attained OTF status in 1980, while France and Belgium reached OTF in 2001 and 2002, respectively (More et al., 2017). Similarly, Australia eradicated bTB from its cattle population in 1997, with no cases reported since 2000 (Cousins & Roberts, 2001; More et al., 2015). Japan, after a long eradication effort, declared itself free from bTB in 2022 (WOAH, 2024). In the U.S., where bTB had been largely eradicated by the 1940s, rare outbreaks linked to infected wildlife have since been reported, with an annual prevalence of seven cases per million cattle (USDA, 2024).

Despite these successes, many regions continue to struggle with bTB. For example, Ireland has reduced herd-level prevalence from 80% in the 1950s to under 5% by 2018 (DAFM, 2018), with plans for complete eradication by 2030 (More, 2019; Ryan et al., 2023). However, in the years following, prevalence began to rise again, and by late 2024, the herd incidence rate had reached 6%

(DAFM, 2024; More, 2024). The sharp increase in prevalence is linked to industry intensification following the removal of milk quotas, resulting in more animals per farm, higher stocking densities, and increased disease transmission (Ryan et al., 2023). In Africa, prevalence rates remain high, with studies estimating infection rates of 3.8% to 5.8% in Ethiopia and up to 10%-13% in Nigeria (Duguma et al., 2017; Ibrahim et al., 2018). In Asia, China has regional areas where over 20% of dairy herds are affected (Zhu et al., 2023).

1.2.1 bTB Prevalence in the UK

In the United Kingdom, Scotland achieved OTF status in 2009. However, England, Wales, and Northern Ireland continue to fight with persistent bTB prevalence, aiming to eradicate the disease at the latest by 2041 (Godfray et al., 2018).

England faces significant challenges, particularly in High-Risk Areas (HRA) in the southwest and west. These regions account for the majority of new infections, with herd prevalence (i.e., the percentage of all herds that are not OTF) exceeding 5%-6% in some areas (AHDB, 2024). The suspension of testing during the 2001 foot-and-mouth outbreak increased an already rising trend in bTB incidence (Vial et al., 2015). Wales has adopted stringent testing protocols since 2010, including annual testing of all herds (Seery et al., 2024). Despite these efforts, wildlife reservoirs, particularly badgers, remain a major obstacle. Northern Ireland continues to enforce rigorous testing but struggles with endemic disease. The bTB incidence rate (i.e., the rate at which new

breakdowns are being detected) in Northern Ireland is 10.41% in 2024, with significant increases observed over the past five years (DAERA, 2024a). The bTB herd prevalence has also risen from 6.93% in 2014 to 12.86% in 2023 (DAERA, 2024b).

The number of new herd incidents across England, Wales, and Scotland has shown a steady decline over recent years (AHDB, 2024) (see Figure 1). Both England and Wales experienced periods of high incidence in the early 2000s through to the mid-2010s. However, a gradual decline in incidence has been observed since these peak years. Scotland has consistently maintained low incidence rates, sustaining its OTF status.

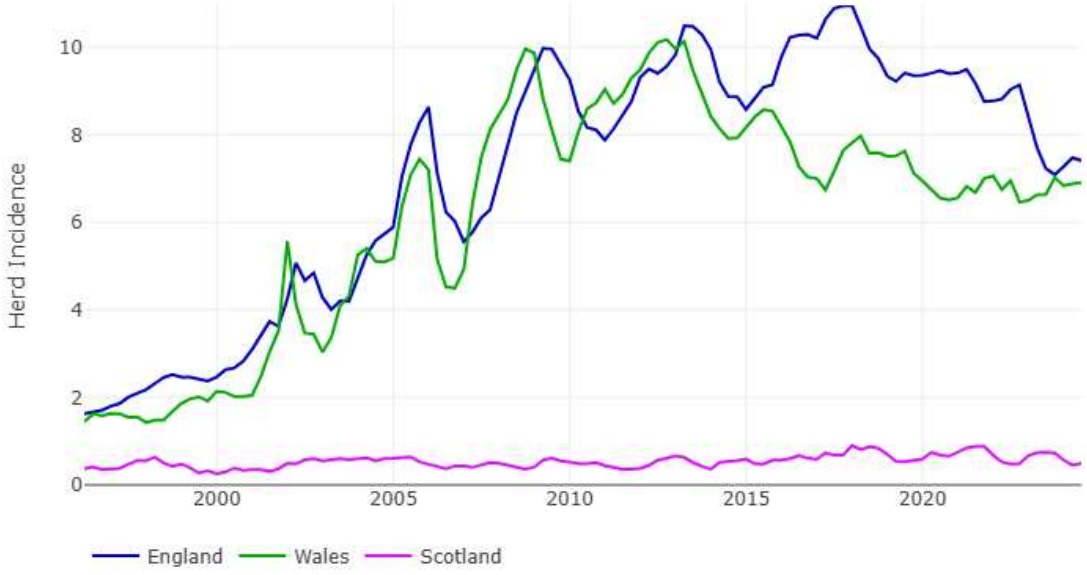

Figure 1: bTB herd incidence per 100 herd years at risk of infection during the year (AHDB, 2024).

Herd prevalence rates in England and Wales mirror the trends observed in incidence, with noticeable peaks around 2005 to 2015, followed by a gradual decline (see Figure 2) (AHDB, 2024). In recent years, prevalence has continued

to decrease, especially in England, where it remains below 5%. Scotland's prevalence has remained low throughout the period.

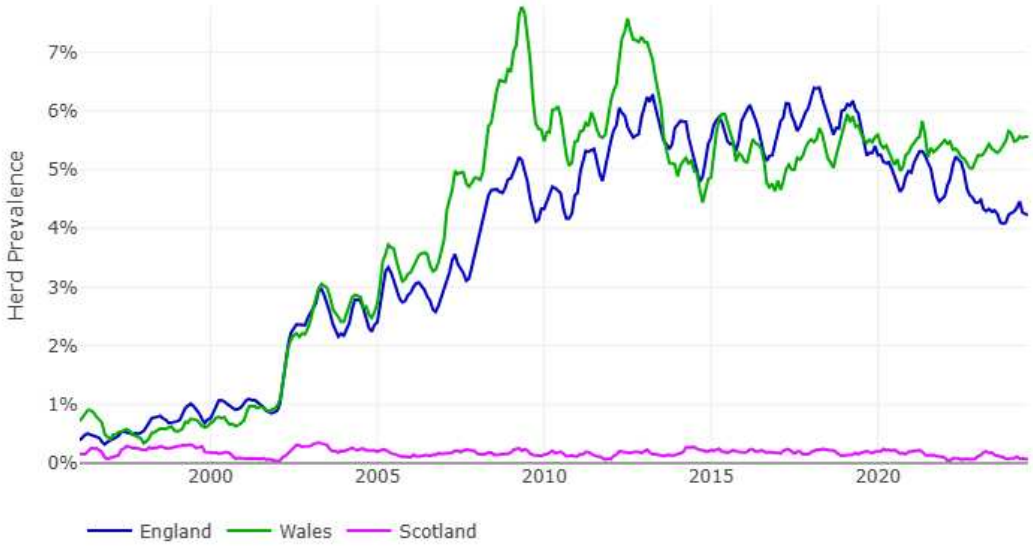

Figure 2: bTB herd prevalence over years (AHDB, 2024).

1.3 Economic Impact of bTB

Globally, bovine tuberculosis affects over 50 million cattle, leading to estimated economic losses of approximately $3 billion annually (Thermo-Fisher-Scientific, n.d.). The economic impact of bTB differs greatly between developed and developing nations. In developed countries, where bTB prevalence is low, costs are primarily driven by eradication programs, with up to 80% of expenses attributed to testing, with majority of being the costs of performing skin tests by veterinarians (Caminiti et al., 2017). In contrast, developing countries face higher bTB prevalence due to limited preventive measures and financial constraints. The primary costs are linked to livestock losses, including reduced

milk and meat production and increased mortality, which significantly impact rural livelihoods and food security (Yahyaoui Azami, 2016).

In the UK, the costs associated with bTB vary widely, influenced by differences in farm characteristics and the nature of disease breakdowns. While dairy herds typically incur higher overall consequential costs compared to beef herds, this is largely due to their larger herd sizes. On a per-head basis, however, costs are generally higher for beef herds. For example, in England, bTB leads to the mandatory culling of over 30,000 cattle annually, imposing a financial burden of approximately £150 million each year on taxpayers and the farming industry (DEFRA, 2020). The compensation prices (in November 2024) for dairy males and for beef males are around £3,255 and £5,149 per animal with ages between 12 and 24 months, although the compensation price is generally calculated by the individual valuation of each animal (DEFRA, 2024). Among the various expenses, testing costs are the most significant, followed by losses in output and the financial impact of movement restrictions (Barnes et al., 2023).

Given these challenges, ongoing research into bTB is essential to enhance our understanding of its epidemiology, pathogenesis, and control. Advances in diagnostics, vaccination, and management practices hold promise for reducing this disease's burden. However, effective control requires efforts across regions, particularly in areas where economic constraints complicate disease management.

1.4 Diagnosis of bTB

Accurate and timely diagnosis is essential for controlling the spread of the disease in cattle populations. Several diagnostic methods are currently in use, each with its own strengths and limitations (De la Rua-Domenech et al., 2006; Schiller et al., 2010).

1.4.1 Post-Mortem Examination

Many countries, including both endemic and OTF regions, conduct routine slaughterhouse screening for bTB lesions as part of strict measures to control and eliminate the disease. Post-mortem examinations (PMEs), carried out by trained veterinarians and inspectors, play a crucial role in confirming the disease and supporting control efforts. These procedures involve visually examining carcasses, organs, and lymph nodes for any visible signs of *M. bovis* related lesions, typically carried out at slaughterhouses or specialized facilities (Carneiro & Kaneene, 2018). Notably, routine slaughterhouse surveillance is crucial in detecting bTB cases, for example, it is estimated that 18-28% of bTB breakdowns were identified through slaughterhouse surveillance in Northern Ireland (Pascual-Linaza et al., 2017). However, the sensitivity (i.e., the ability to correctly identify animals that are truly infected) of PMEs for detecting bTB lesions is relatively low, for example, a study in Australia found that slaughterhouse inspections failed to detect bTB lesions in 47% of infected cattle, highlighting the limitations of this method (Corner et al., 1990).

Nevertheless, routine slaughterhouse surveillance remains a cost-effective method for monitoring large numbers of cattle for bTB lesions (Corner, 1994).

After visual inspections, bacterial culture is widely regarded as the gold standard for the definitive diagnosis of bTB as it involves the isolation of *M. bovis* bacteria from tissue samples collected post-mortem (De la Rua-Domenech et al., 2006). Despite its accuracy, bacterial culture has significant limitations: it is time-consuming due to the slow growth rate of *M. bovis*. Additionally, the process is expensive and requires specialized laboratory facilities (Gormley et al., 2014). Because of these constraints, bacterial culture is primarily used to confirm bTB infections rather than for routine screening.

Polymerase Chain Reaction (PCR) testing has been an alternative method for diagnosing various infectious diseases since its development in the 1980s. In the context of bTB, PCR has been utilized globally to detect *M. bovis* directly from clinical samples (De la Rua-Domenech et al., 2006; Courcoul et al., 2014). In various countries, PCR testing has been integrated into bTB control programs to improve diagnostic accuracy and speed. Unlike traditional bacteriological culture, which can take 6 to 22 weeks to confirm the presence of *M. bovis*, PCR testing provides results within approximately three weeks, significantly accelerating diagnostic timelines. This reduction in processing time enables faster decision-making regarding herd movement restrictions and disease control interventions (Aman et al., 2017; APHA, 2024).

In GB, PME is conducted either by the Animal Plant and Health Agency (APHA) or by meat inspectors at abattoirs (APHA, 2021; Payne et al., 2024). These examinations are similar in the Republic of Ireland and Northern Ireland (O'Hagan et al., 2019; Byrne et al., 2020), aiming to identify visible lesions of bTB and may include further laboratory testing of tissue samples to confirm the presence of *M. bovis* (Payne et al., 2024). These lesions, often found in the respiratory tract, indicate advanced disease. However, visible lesions are not always present, particularly in early or localized infections; for example, only 36% of infected animals showed lesions at slaughter in 2019 in England (TbHub, 2020). The size and number of lesions may vary based on infection duration and transmission route (Pollock & Neill, 2002). APHA also conduct bacteriological culture of tissue samples to identify the specific strain of *M. bovis* through DNA typing or sequencing (Payne et al., 2024) and PCR tests (Morris et al., 2023) to confirm the presence of *M. bovis*. The PCR test was introduced in Great Britain by the APHA in March 2022 and represents a significant advancement in diagnostic methodologies. Initially, its use was focused on samples from slaughterhouse bTB cases and cattle with suspicious TB lesions identified during routine meat inspections (i.e., animals sent for commercial slaughter that showed suspicious lesions during standard inspection). Currently, the scope of the PCR test was expanded to include post-mortem samples from TB-positive cattle identified through tests such as skin and interferon-gamma, as well as direct contacts and inconclusive reactors that had been slaughtered.

1.4.2 Field Tests

The tuberculin skin test (TST) has been a cornerstone in the diagnosis of bTB in cattle for over a century (Good et al., 2018). Developed in the late 19th and early 20th centuries, the test involves the intradermal injection of purified protein derivative (PPD) tuberculin into the skin. In infected cattle, the injection induces a delayed-type hypersensitivity reaction, resulting in localized swelling at the injection site, which is measured after 48 to 72 hours to determine infection status (De la Rua-Domenech et al., 2006).

There are two main types of TSTs used globally: the Single Intradermal Test (SIT) and the Single Intradermal Comparative Cervical Tuberculin (SICCT) test. In the SIT, bovine PPD alone is injected, while, the SICCT test uses both bovine PPD and avian PPD to improve specificity (i.e., the ability of the test to correctly identify animals that do not have bTB) by distinguishing between responses caused by *M. bovis* infection and exposure to environmental mycobacteria (De la Rua-Domenech et al., 2006). The choice of skin test used depends largely on the prevalence of bTB and the likelihood of exposure to environmental mycobacteria in a given region. SIT is commonly used as the primary screening test across continental Europe. The SICCT test, offering improved specificity, is widely used in countries with higher bTB prevalence, such as Great Britain and Ireland, as part of rigorous control and eradication programs (Allen et al., 2018).

The widespread adoption of the TST is primarily due to its simplicity and suitability for large-scale screening, making it a critical tool for bTB control in

cattle worldwide and it remains the most practical and widely used diagnostic test (RABDF, 2024).

In GB, the main diagnostic tool for bTB detection is the SICCT test (De la Rua-Domenech et al., 2006; Griffin et al., 2023). It is an *in vivo* test and has been used since 1942 (Monaghan et al., 1994; RABDF, 2024). The SICCT test generally has a high specificity above 97%, but the sensitivity of SICCT can vary between 50-80% (Nuñez-Garcia et al., 2018), leading to false negatives, particularly in the early stages of infection or in animals with compromised immune systems (Goodchild et al., 2015). Although SICCT is commonly used as it allows rapid *in vivo* diagnostics, it can be time and labour-intensive, since it requires two visits to the herd (for injection and assessing the reaction), making it challenging and costly (De la Rua-Domenech et al., 2006).

1.4.3 Laboratory Tests

The Interferon Gamma (IFN-γ) assay is a blood-based test that measures the production of interferon-gamma (IFN-γ) by T cells in response to stimulation by bTB antigens. Developed in Australia in the late 1980s, the assay has been approved under EU legislation for use in cattle since 2002 (De la Rua-Domenech et al., 2006). The IFN-γ assay is used alongside the TST to maximize detection rates of bTB infected animals, particularly in cattle herds experiencing chronic bTB breakdowns. This assay offers several advantages, including higher sensitivity compared to SICCT, which allows for detecting early infections. The IFN-γ assay requires only one visit to the herd for blood sampling, making it easier than SICCT. It also increases the detection rate,

identifying infected animals that might not react to the SICCT, thus improving overall detection in a herd (Coad et al., 2008; Goodchild et al., 2015). However, the IFN-γ assay is more expensive than SICCT and requires specialized laboratory equipment and trained personnel, which can be a limiting factor in some settings (Praud et al., 2019). While generally more sensitive (83-92%), the specificity can be lower than SICCT (83-97%), leading to more false positives (Bisschop et al., 2023). Additionally, blood samples must be processed quickly, which can be challenging in remote areas (TBHub, 2023). The IFN-γ assay is widely regarded as a valuable supplementary test to the TST rather than a standalone diagnostic tool. It is particularly useful in high-risk herds, where its ability to detect infections helps reduce transmission and supports bTB control programs (Gormley et al., 2006; Coad et al., 2008). Countries such as Great Britain and Ireland have adopted the IFN-γ assay to complement TST and improve overall detection rates (Lahuerta-Marin et al., 2015; Clegg et al., 2017).

Antibody-based serological tests, such as enzyme-linked immunosorbent assays (ELISA), have emerged as ancillary tools to complement traditional tests, like the TST and IFN-γ, particularly in identifying animals missed during advanced stages of bTB. During the early stages of infection, TST and IFN-γ are effective in detecting infected cattle. However, as the disease progresses and cell-mediated immune responses decline (a phase known as anergy), the humoral immune response involving antibodies becomes more prominent,

offering a window for serological detection (Ritacco et al., 1991; Vordermeier et al., 2004).

The IDEXX *M. bovis* test and the Enferplex TB test are two commercially available antibody based platforms. The IDEXX ELISA detects antibodies against the *M. bovis* antigens (Waters et al., 2011). The Enferplex test is a multiplex assay capable of detecting antibodies against multiple antigens, enhancing diagnostic flexibility (Whelan et al., 2008; Whelan et al., 2010). These tests are particularly valuable in chronic bTB breakdown herds or cases where skin test negative animals exhibit advanced disease and produce high levels of *M. bovis* specific antibodies (Pollock & Neill, 2002). While antibody-based tests cannot replace TST or IFN-γ assay, they play a crucial role as supplementary tools in chronic and advanced infections (Vordermeier et al., 2004; De la Rua-Domenech et al., 2006).

1.5 Non-Genetic bTB Control Strategies

Controlling bTB is challenging due to its complex epidemiology involving livestock and wildlife reservoirs. Globally, the control of bTB is a complex and ongoing challenge, requiring a multifaceted approach that incorporates various strategies such as surveillance, testing, culling, vaccination, and biosecurity measures. These strategies vary significantly between countries, depending on factors like the prevalence of the disease, the role of wildlife reservoirs, and the resources available for control efforts (White et al., 2008; VanderWaal et al.,

2017; Arnot & Michel, 2020). Effective control is critical for public health, animal welfare, and economic stability in the agricultural sector.

1.5.1 Surveillance and Culling

In the GB, the control of bTB is of particular importance due to the significant impact of the disease on the cattle industry and the associated economic and animal welfare concerns. The governments' bTB eradication strategies rely on strict surveillance and culling measures for cattle herds (DEFRA, 2018). Surveillance is conducted through routine herd testing (SICCT), with the frequency ranging from six months to four years, depending on the herd's location within assigned bTB risk areas (see Figure 3). In 'Low Risk' areas, testing is required every four years, while in 'Edge' areas, it occurs every six or twelve months. Herds in 'High Risk' areas undergo testing every six months (Salvador et al., 2018; APHA, 2023a). The SICCT test involves injecting two types of tuberculin (avian and bovine) into the skin and comparing the reaction size at both injection sites. A positive result, or 'reactor', is declared when the reaction to bovine tuberculin exceeds the avian reaction by more than 4mm (or 2mm in high-risk areas). If the bovine reaction is slightly larger than the avian reaction but does not meet the threshold for a reactor, the test is labelled inconclusive. A bTB breakdown is declared if a herd has at least one cow with one positive result or two consecutive inconclusive results (animals with inconclusive results are re-tested after two months). Inconclusive animals are considered higher risk, as they have a significantly greater likelihood of

becoming reactors on follow-up tests (Clegg et al., 2011). This triggers immediate culling of reactors (or repeated inconclusives), as well as movement restrictions and withdrawal of the herd's OTF status, and mandatory SICCT testing is conducted every 60 days. Animals with positive or inconclusive test results are immediately sent for slaughter, where further examinations for bTB lesions are conducted (De la Rua-Domenech et al., 2006). This rigorous testing continues until all animals test negative in two consecutive tests, after which the herd regains its OTF status and movement restrictions are lifted.

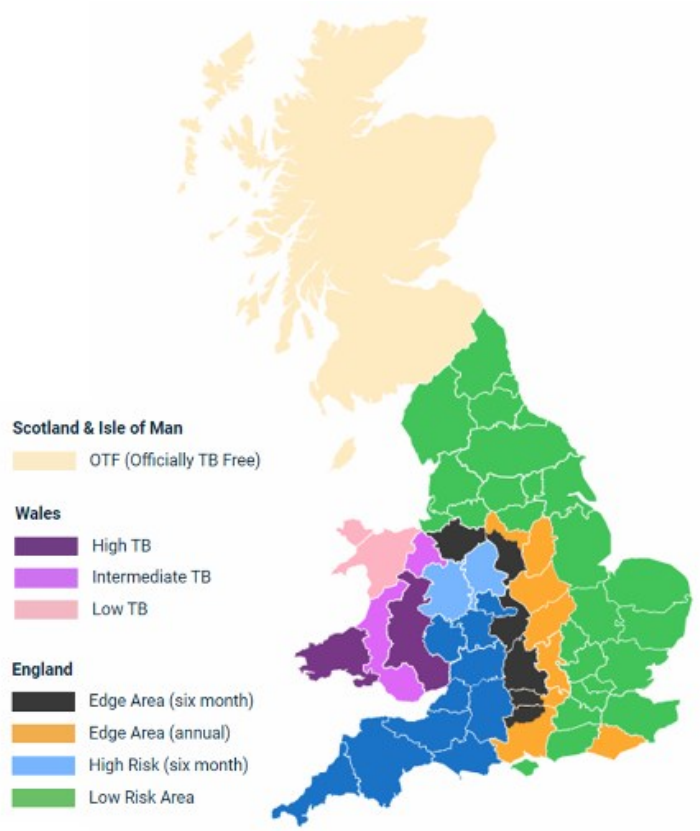

Figure 3: bTB risk areas in England, Wales and Scotland (DEFRA, 2018).

The stringent culling regime is seen as a necessary measure to control the disease, and it has contributed significantly to reducing bTB prevalence in many

regions (Krebs, 1998). However, the effectiveness of this approach has been limited in areas with high densities of wildlife reservoirs, particularly badgers. For example, in high-risk areas such as Southwest England and Wales, average badger densities can reach up to 5.98 per km^2, whereas in low-risk areas, average densities may be as low as 0.26 badgers per km^2 (Judge et al., 2017). In regions with high wildlife densities, the persistence of bTB in wildlife has complicated efforts to eradicate the disease in cattle, leading to ongoing challenges for farmers and policymakers (DEFRA, 2018; Mitchell et al., 2022).

One of the most controversial aspects of bTB control in the GB has been the culling of badgers, which are known wildlife reservoirs of *M. bovis*, and culling is intended to reduce the transmission of the disease from badgers to cattle. However, the effectiveness of badger culling has been widely debated (Donnelly et al., 2007; Donnelly & Nouvellet, 2013). Some studies suggest that badger culling can reduce bTB incidence in cattle, particularly in areas with high badger populations and where the disease is prevalent (Donnelly et al., 2006; Downs et al., 2019). However, others indicate that it may disrupt badger populations and increase disease spread through a process known as perturbation (Tuyttens et al., 2000; Macdonald et al., 2006; Langton et al., 2022). For example, GB based researchers predicted that even selective removal of TB positive badgers could result in detrimental perturbation effects based on simulation modelling (Bielby et al., 2014). However, the experience in Ireland and Northern Ireland presents a contrasting perspective. In Ireland, selective culling of TB-positive badgers has shown long-term beneficial effects

in reducing disease, with no observed perturbation effect (More et al., 2007; Corner et al., 2008; Olea-Popelka et al., 2009). In fact, culling has been a part of the Irish disease control strategy and has demonstrated measurable positive impacts lasting up to ten years. In Northern Ireland, selective culling approaches have similarly shown no evidence of perturbation (O'Hagan et al., 2021; Allen et al., 2022). More recently, the development of vaccination strategies has provided an additional approach alongside selective culling of TB-positive badgers, aiming to achieve a more sustainable balance between wildlife conservation and disease control (Ryan et al., 2023; Byrne et al., 2024).

1.5.2 Vaccination

Vaccination is another important strategy for bTB control, aiming to reduce the incidence of infection in both cattle and badger populations. The Bacillus Calmette-Guérin (BCG) vaccine, the only licensed anti-TB vaccine in humans, has been shown to be effective in both cattle and badgers, although it does not confer full protection (Buddle et al., 2018; Hope et al., 2023). Application of the BCG vaccine in cattle, however, faces challenges, particularly due to current diagnostic limitations, that prevent it from being licensed. The existing skin test used for bTB detection cannot distinguish between vaccinated and infected animals, making it difficult to implement widespread cattle vaccination without compromising disease surveillance efforts (Fromsa et al., 2024). However, ongoing research and field trials currently evaluate the safety of the BCG vaccine, as well as the safety and effectiveness of a differential diagnostic test

called DIVA (Differentiating Infected from Vaccinated Animals). This test could facilitate the wider use of cattle vaccination as part of a strategy to control bTB (Buddle et al., 2018; APHA, 2023b). If trials are successful, cattle vaccination could offer a valuable tool in reducing bTB prevalence in the long term, complementing other control measures.

Badger vaccination, using the BCG vaccine, has been trialled in various parts of the UK and Ireland with the goal of reducing transmission to cattle (Carter et al., 2012; Woodroffe et al., 2024). The efficacy of badger vaccination has been assessed through experimental and field trials, showing vaccination efficacies ranging from 36% to 84% (Martin et al., 2020; Byrne et al., 2024). While it is less controversial than culling, its impact on bTB incidence is still under study and challenges remain in terms of the logistics of vaccinating wild populations on a large scale (Godfray et al., 2018; Ryan et al., 2023). Some studies also showed that badger vaccination, when combined with cattle test-and-removal and movement restrictions, is insufficient to eradicate bTB (Chang et al., 2024a, 2024b). They also indicated that cattle-to-cattle transmission remains the predominant source of new infections at the individual animal level. This underscores the need for additional interventions to reduce cattle-based transmission (van Tonder et al., 2021; Akhmetova et al., 2023).

1.5.3 Biosecurity Measures

In addition to testing, culling, and vaccination, biosecurity measures also play a critical role in preventing the spread of bTB within and between farms (Sibley, 2024). These measures include practices such as controlling farm access to minimize contact with potentially infected animals, managing wildlife interactions to reduce the risk of transmission from wildlife to cattle, and ensuring good herd management practices, including regular health checks and maintaining hygiene standards (Renault et al., 2021). In the UK, farmers are strongly encouraged to implement reasonable biosecurity practices as a key component of bTB control. One particularly beneficial strategy is the adoption of risk-based approaches, which aim to minimise transmission by tailoring controls to the specific risk level of each herd (More, 2024). This includes herd management protocols whereby herds are classified as high, medium, or low risk based on their infection history. The newly derestricted herds, especially those following major breakdowns, start at high risk and are gradually reclassified after successive negative tests. Risk-based cattle trading rules further reinforce this by ensuring animals are only sold to herds of the same or higher risk category, reducing the chance of spreading infection. Additionally, regionalisation which is the division of countries into zones based on infection levels, enables more efficient resource allocation and targeted intervention, and has been integral to eradication efforts in different countries (More, 2024). While biosecurity measures can be highly effective in reducing the risk of bTB transmission, their implementation varies widely across farms,

often due to differences in farm size, resources, and the level of awareness among farmers (Godfray et al., 2018).

1.6 Genetic bTB Control Strategies

bTB remains a persistent challenge for the agricultural industry, highlighting the necessity for ongoing exploration of supplementary control measures that can effectively complement existing eradication efforts (Godfray et al., 2018; Banos, 2023). The complexity of eradicating bTB highlights the need for diverse and innovative approaches that target different aspects of disease management (O'Hare et al., 2014).

Animals' responses to *M.bovis* challenge vary widely and this difference in response may be partly influenced by genetic factors. For bTB control, selecting animals with stronger genetic bTB resistance or a reduced ability to spread the disease once infected could help limit the spread of bTB in future generations (Godfray et al., 2018).

1.6.1 Breeding for Resistance

Breeding cattle for enhanced resistance to bTB represents a promising long-term and complementary strategy for disease control. This approach is driven by growing evidence of genetic variation in cattle's resistance to bTB and its potential to reduce disease prevalence and severity (DEFRA, 2018).

Simulation models have predicted promising outcomes with the use of this selection approach (Raphaka et al., 2018; Banos, 2023). Specifically, after five generations of selective breeding focused on sires with high genetic merit for bTB resistance, the risk of a disease outbreak could be reduced by as much as 50%. Moreover, these models predict that, following just one generation of selective breeding, the average duration of bTB outbreaks could be shortened by 5-10%, and the severity, in terms of the number of animals testing positive, could be reduced by 11-17% (Raphaka et al., 2018; Banos, 2023).

Several studies have assessed the genetic variation in resistance to bTB across various cattle populations and countries, employing diverse statistical models and trait definitions (Petukhov et al., 1998; Bermingham et al., 2009; Allen et al., 2010; Brotherstone et al., 2010; Richardson et al., 2014; Banos et al., 2017; Ring et al., 2019; Callaby et al., 2020). The heritability of bTB resistance in cattle has been investigated, which measures the proportion of variation in resistance attributed to genetic factors. Estimates generally range from 0.07 to 0.34 (Bermingham et al., 2009; Brotherstone et al., 2010; Raphaka et al., 2017). These findings indicate that genetic selection for increased resistance is feasible, offering a potentially valuable tool in the eradication of bTB. These investigations have primarily relied on extensive field data paired with detailed animal pedigree information. Despite variations in datasets and trait definitions, these studies consistently highlight the genetic basis of bTB resistance (Allen et al., 2010; Banos, 2023).

In the UK, the progress in identifying genetic resistance to bTB has been largely facilitated by using national surveillance datasets, which include records of bTB test results for individual animals. These datasets are combined with information routinely collected for national genetic evaluations, allowing researchers to gain deeper insights into the genetic factors associated with bTB resistance (Banos et al., 2017). By combining data from multiple sources, such as the SICCT test, post-mortem examination of bTB lesions, and *M. bovis* culture tests, researchers have been able to characterise the bTB status of individual animals across different time points. This combination of data sources has enabled the development of a resistance phenotype, which refers to the individual propensity to not be positive in a bTB test (Tsairidou et al., 2016; Banos et al., 2017).

The UK dairy industry introduced the "TB Advantage" selection index in 2016 (Banos et al., 2017). This index was developed to help farmers select breeding bulls with higher genetic resistance to bTB, ultimately aiming to enhance the overall resistance of dairy herds across the country (BCBC, 2016). By incorporating this selection index into breeding programs, the goal is to accelerate the decline in bTB incidence and severity, complementing the progress achieved by other eradication strategies. Similar evaluations are also available in Ireland for both dairy and beef cattle, with evaluations conducted by the Irish Cattle Breeding Federation since 2019 (ICBF, 2024).

Although current evidence suggests that resistance is a complex trait controlled by many genes and influenced by a strong environmental component

(indicative of a polygenic architecture) (Richardson et al., 2016; González-Ruiz et al., 2019; Ring et al., 2019), the integration of genome-wide data into genetic evaluations provides additional opportunities to enhance selection accuracy, particularly in scenarios where pedigree information alone may be limited (Banos, 2023). Genomic studies have identified Single Nucleotide Polymorphisms (SNPs) and genomic regions associated with bTB resistance, with estimates indicating that these regions explain 5% to 8% of the genetic variance for resistance traits (Bermingham et al., 2014; Raphaka et al., 2017). Furthermore, genome-wide analyses have identified candidate genes and biological networks underlying resistance, offering deeper insights into the complex genetic architecture of bTB resistance (Bermingham et al., 2014; Tsairidou et al., 2014; Raphaka et al., 2017; Callaby et al., 2020). However, the number of genomic datasets is often smaller compared to traditional pedigree datasets, which can sometimes lead to inflated estimates of heritability (Banos, 2023), also known as the Beavis effect (Xu, 2003). Nevertheless, they remain as a valuable tool for identifying marker effects and refining breeding strategies.

1.6.2 Breeding for Infectivity

While genetic selection for resistance to bTB is a promising method to reduce bTB in the long-term, relying solely on resistance based breeding may not be sufficient to eliminate bTB within a reasonable timeframe (Tsairidou et al., 2018; Banos, 2023). To achieve faster progress, additional breeding objectives need to be explored. One promising approach involves incorporating host traits that influence disease transmission alongside resistance (Godfray et al., 2018).

Reducing the infectiousness of a host has always been a crucial part of minimizing disease transmission (Geenen et al., 2004; Lloyd-Smith et al., 2005; Tsairidou et al., 2018). Infectiousness is a measure of how effectively a disease spreads within a population. It is determined by three key factors: the contact rate between infected and non-infected individuals, the propensity of infected individuals to transmit the disease upon unit contact (i.e., infectivity), and the duration of the infectious period (Lipschutz-Powell et al., 2014). In the case of bTB, control measures such as movement restrictions and test-and-cull policies significantly reduce both the contact rate and the infectious period. Movement restrictions limit interactions between infected and non-infected individuals, while test-and-cull strategies reduce the duration of infection by removing detectable infected animals. Although vaccination could potentially reduce infectivity, there is currently no licensed bTB vaccine in cattle (APHA, 2023b). Field trials indicated that the protection provided by the BCG vaccine is limited and it does not fully prevent infection, with its overall protective efficacy estimated at approximately 25% (Hope et al., 2023).

Infectivity refers to the capacity of an infected individual to transmit the disease to others within the population (Read & Taylor, 2001; Geenen et al., 2004; Keeling & Danon, 2009; Brooks-Pollock et al., 2015). Unlike resistance, which concerns an individual's likelihood of becoming infected or developing disease, infectivity pertains to how likely an infected host can spread the disease. These two traits together shape the dynamics of disease outbreaks and influence the speed and magnitude of infection spread within a herd.

To date, there is limited empirical evidence for host genetic variation in bTB infectivity (Tsairidou et al., 2018). Studies have shown that not all animals are equally infectious, and some individuals, known as "super-spreaders", contribute disproportionally to the spread of bTB (O'Hare et al., 2014; Fielding et al., 2021). In addition, recent literature has compiled evidence for substantial phenotypic variation in cattle bTB infectivity, considering the differences in the shedding patterns of *M. bovis* (Tsairidou et al., 2018). Studies in other species indicate that host infectivity is partly genetically determined (Geenen et al., 2004; Doeschl-Wilson et al., 2018; Anacleto et al., 2019). As such, if genetic variation for bTB infectivity in cattle can be identified and accurately estimated, selecting for infectivity alongside resistance could significantly speed up reductions in bTB prevalence and transmission rates (Tsairidou et al., 2018; Tsairidou et al., 2019). Specifically, identifying and selectively breeding these super-spreaders out of the population could significantly reduce overall infection rates (Lloyd-Smith et al., 2005; Bishop & Woolliams, 2014). However, the success would depend on factors such as heritability, selection intensity, prediction accuracy, and the genetic correlation between infectivity, resistance

and other economically important traits currently in use within breeding programs (Banos, 2023).

Despite its potential, infectivity presents measurement challenges as it cannot be directly observed, and disentangling it from susceptibility using conventional genetic models is difficult (Lipschutz-Powell et al., 2014; Bijma et al., 2022).

Several methods have been proposed to estimate the genetic basis of infectivity using epidemiological data. These require integration of genetic and epidemiological modelling approaches. Epidemiological models that incorporate genetic data can help explain how genetic variation in susceptibility and infectivity translates into changes in disease prevalence and outbreak duration. For instance, reducing the basic reproductive ratio (R_0) below one is critical to stopping epidemic spread (i.e., when R_0 is less than one, the epidemic will die out; when it exceeds one, widespread outbreaks can occur) (Diekmann et al., 1990). Theoretical studies demonstrated that genetic variation in both, host susceptibility and infectivity contribute to R_0 and could be targeted to reduce it (Lipschutz-Powell et al., 2012; Anche et al. (2014).

Approaches to estimate genetic parameters for infectivity involve treating infectivity as an indirect genetic effect within a Generalized Linear Mixed Model (GLMM) framework (Lipschutz-Powell et al., 2012; Anche et al., 2014; Biemans et al., 2017; Biemans et al., 2019). Although these models are practical and widely used, they rely on a number of assumptions and simplifications, which can introduce biases in genetic estimates. They also require accurate and frequent data on individual infection status, which can be difficult to obtain in

real-world settings (Lipschutz-Powell et al., 2012; Biemans et al., 2017; Biemans et al., 2019).

More sophisticated techniques, such as incorporating genetic-epidemiological models within hierarchical Bayesian frameworks, have also been developed to estimate genetic parameters for infectivity without relying on oversimplifications (Anacleto et al., 2015; Doeschl-Wilson et al., 2018; Anacleto et al., 2019; Pooley et al., 2020; Pooley et al., 2024). This approach offers greater flexibility in dealing with uncertainty and missing data. Bayesian methods, such as Markov Chain Monte Carlo (MCMC), can incorporate different data sources and model structures to estimate genetic parameters for infectivity. These advanced methods are more adaptable, particularly when data are incomplete or irregular, which is often the case in practical livestock management scenarios. These methods have also shown promising results in experimental studies, revealing significant genetic variation in infectivity similar to that of resistance (Prentice et al., 2022). However, adapting these methodologies to manage the complexity and volume of national bTB field and genetic datasets requires extensive validation and methodological adjustments (Tsairidou et al., 2018).

1.7 Thesis Aim, Objectives and Outline

The overriding aim of this PhD thesis is to investigate the presence of genetic variation in bTB infectivity of dairy cattle in Great Britain using field data from national bTB surveillance. The following specific objectives are set in this regard:

1. Develop and explore novel phenotypes for dairy cattle infectivity and examine factors affecting them (Chapter 2)
2. Detect genetic variability in the novel infectivity phenotype and estimate its variance components through the use of different statistical models (Chapter 3)
3. Assess the implications of incorporating infectivity into breeding programs on future bTB prevalence and investigate the correlations between the novel phenotype and other economically important traits (Chapter 4)

This thesis is divided into five chapters. Chapter 1 presents this introduction and literature review on bTB, transmission pathways, pathogenesis, prevalence, diagnosis, and control strategies. Chapter 2 explores bTB field data, which is already assembled for TB Advantage, and introduces the concept of the "index case approach" to define novel bTB infectivity phenotypes. Linear and generalized linear mixed models (GLMMs) implemented in frequentist approaches are employed to obtain first estimates for influencing factors and variance components of the novel phenotypes. Chapter 3 uses Markov Chain Monte Carlo (MCMC) techniques to fit four types of GLMMs to obtain and

compare genetic variance estimates for bTB infectivity. Chapter 4 considers the implications of incorporating infectivity into breeding programs by assessing the potential epidemiological impact of selecting for reduced infectivity and examining correlations of sire EBVs for the novel infectivity phenotype with sire EBVs for resistance to bTB, as well as with EBVs for other economically important traits. Chapter 5 concludes the thesis by summarizing the key findings and provides a general discussion.

The findings of this thesis are expected to make a novel contribution to genetic bTB control strategies by supporting the integration of infectivity into multi-trait selection schemes. This could help simultaneously improve cattle resistance to bTB and reduce its transmission, with potential applications in breeding programs.

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Chapter 2

Exploring bTB infectivity at the phenotypic and genetic level

2.1 Introduction

Defining the infectivity phenotype of bovine tuberculosis (bTB) is crucial for improving selective breeding programs that aim to reduce disease spread. Infectivity refers to an individual animal's capacity to spread infection, yet direct measurements of this trait remain challenging (Knap & Doeschl-Wilson, 2020; Banos, 2023). Field data often lack clear transmission pathways, making it difficult to determine precisely who-infected-whom. Additionally, not all transmission events are observed, further complicating efforts to assess infectivity (Tsairidou et al., 2018).

Infectivity phenotypes have been estimated through individual shedding rates (McCorry et al., 2005; Charpin et al., 2012). However, this approach is only feasible in limited scenarios and is impractical for the large-scale data collection required for breeding programs (Tsairidou et al., 2018). Nevertheless, shedding is not the only phenotype for assessing infectivity. Recent advancements in inference methods have enabled the estimation of genetic variation in infectivity by monitoring infection progression across herds over time (Anacleto et al., 2015; Anche et al., 2015; Biemans et al., 2017). These methods used

longitudinal data, including the infection status of individuals, to disentangle genetic factors influencing both resistance and transmission potential.

In the context of bTB, data from the UK national eradication scheme provides a systematic collection of repeated SICCT (Single Intradermal Comparative Cervical Tuberculin) test results from a wide range of herds with related animals, creating an opportunity to evaluate infectivity. Although exact infection times are not directly observed, repeated testing allows to infer these times (Anacleto et al., 2015; Pooley et al., 2020; Pooley et al., 2022; Pooley et al., 2024). Nonetheless, the lack of a clearly defined infectivity phenotype limits the potential of advanced genomic tools and statistical models, highlighting an urgent need to focus on defining and measuring this critical trait for effective disease control (Banos, 2023).

Several non-genetic factors may influence the infectivity phenotype, and understanding these factors is crucial. One of the factors is the age of the infected animal since younger cattle, particularly those aged 12 to 36 months, show a higher risk of infection (Cadmus et al., 2010; Brooks-Pollock et al., 2013; Broughan et al., 2016). The size of the herd may also play an important role in bTB infectivity. Larger herds have been found to be associated with a higher risk of disease persistence, likely due to increased opportunities for close contact between animals, which may facilitate the spread of infection (Reilly & Courtenay, 2007; Milne et al., 2020). Geographical differences may also influence bTB spread. Studies have shown that environmental conditions and regional farming practices may differ and influence disease spread (White &

Benhin, 2004; Broughan et al., 2016). Seasonal and temporal factors may also affect bTB infectivity. Factors like the year and season when an outbreak starts are crucial because seasonal variations in environmental conditions, such as temperature and moisture, may influence the survival and spread of *M. bovis* (Broughan et al., 2016). In the UK and Ireland, cattle are typically housed indoors during the colder months, often in poorly ventilated facilities. This increase in stocking density may facilitate the transmission of bTB by promoting closer contact and reduced airflow. Furthermore, some studies suggested that lactating and pregnant cows may have different susceptibilities to bTB, which indicates that lactation length may be another factor influencing disease transmission (Broughan et al., 2016; Mellado et al., 2021). Although these findings indicate that age, herd size, location, and temporal factors influence susceptibility, persistence, and risk of infection, more research is needed to fully explore whether these factors also affect bTB infectivity.

The primary objective of this chapter is to explore novel phenotypes for bTB infectivity and examine factors affecting them.

2.2 Materials and Methods

2.2.1 Data

The data used in this chapter were obtained from the national genetic evaluations for bTB resistance in dairy cattle (Banos et al., 2017), encompassing Great Britain (GB) herds that experienced bTB confirmed

breakdowns between 2000 and 2022. The data had been originally derived from different sources (Banos et al., 2017), including the Animal and Plant Health Agency (APHA), the British Cattle Movement Service (BCMS), milk recording databases, and the Edinburgh Genetic Evaluation Services (EGENES). The combined data were provided by EGENES under agreement. The data consist of routinely collected tuberculin skin test (SICCT test) and post-mortem examination records of dairy cattle. Skin tests, conducted on an approximately bi-monthly basis during bTB breakdown periods, were used to define infection status, which helps to classify animals as healthy or infected. In addition, national pedigree information from EGENES was merged to link animals with their sires and dams, providing the basis for genetic analysis. The data contain one record per animal per bTB breakdown where a breakdown refers to bTB being disclosed in an unrestricted cattle herd within the reporting period, and for each animal in a breakdown, the following records were provided.

Animal ID: A unique identifier of an animal.

Sire of Animal ID: A unique identifier of an animal's sire.

Breakdown Dates and Duration: The start date, end date, and duration of a bTB breakdown.

CPHH Code: The unique code representing the herd's County, Parish, Holding, and Herd number.

Breed and Age Information: Breed of the animal and its age at the start of the breakdown, recorded in days.

Ante-Mortem and Post-Mortem Results: Skin test, Slaughter Status, Lesion, and Culture results of an animal.

Lactation Number: The number of times a cow has calved.

Pedigree Data: Contain records for each animal, linking an Animal ID with its Sire ID and Dam ID.

Infection Status: Coded as a binary trait (healthy or infected). The classification of infection status relies on a combination of skin test and post-mortem examinations. Animals were defined as infected (i.e., bTB case) if either the skin test or the post-mortem examination resulted in a positive test result. In other words, this includes animals that reacted to the skin test with post-mortem confirmation of visible bTB lesions or a positive *M. bovis* culture. Additionally, animals were also defined as infected if they were skin test reactors, regardless of their post-mortem examination results. Also, the animals with a negative skin test or inconclusive skin test results were defined as infected if they showed positive post-mortem examination results, thus aiming to capture possible false negatives. On the other hand, healthy animals were defined as non-reactors to the skin test or slaughtered animals without bTB lesions and with confirmed negative *M. bovis* culture results. This definition of infection status was described in the study of national genetic evaluation for bTB resistance (Banos et al., 2017).

The dataset comprised over 43,000 official bTB breakdowns across Great Britain from 2000 to 2022, covering 6,150,915 animal records across 15,277

unique herds. Pedigree information included 13,549,206 records spanning multiple generations.

2.2.2 Infectivity phenotype

In this thesis, the “index case approach” is introduced to define bTB infectivity phenotypes. This approach assumes that, once an initial infected animal, defined as the index case, is detected, the subsequent infections within the early stages of a bTB breakdown (defined as secondary cases) are most likely to have originated from this primary infected animal. By analysing patterns of spread following the detection of index cases, variations in infectivity among different individuals may be better understood.

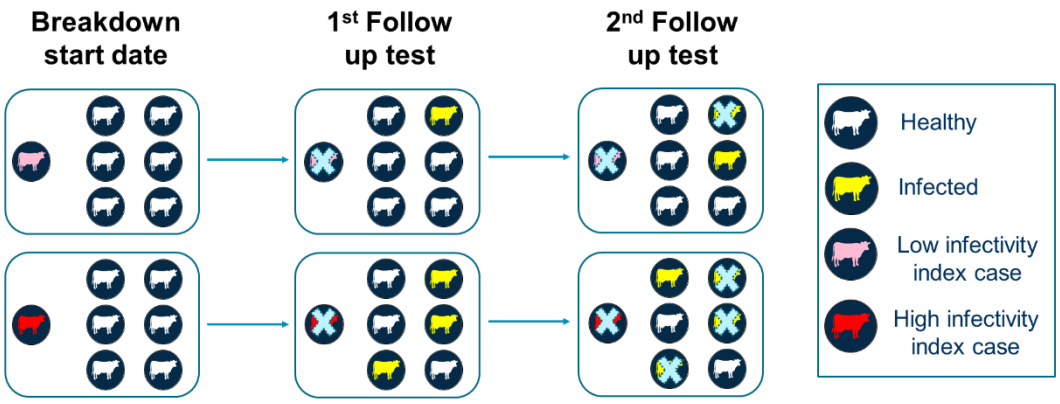

Figure 1: Index case approach. The breakdown start date refers to the date on which the initial test-positive animals triggered the declaration of a bTB breakdown within the herd. Follow-up tests were conducted approximately 60 and 120 days after the breakdown start date (±30 days), representing intervals of roughly 60 days between each test.

Figure 1 provides a conceptual representation of the index case approach, illustrating two distinct bTB breakdowns, each initiated by a different index case. The core idea here is that if the index cases differ in their infectivity, then the

resulting breakdowns may vary in the rate at which new bTB cases are detected. For instance, an index case with high infectivity would be expected to produce, on average, more secondary cases compared to an index case with low infectivity. While individual variations in outcomes may arise due to the inherently stochastic (random) nature of disease transmission or due to external environmental factors, observing consistent patterns across genetically related index cases may provide evidence of a genetic component influencing infectivity. Therefore, the number of secondary cases associated with an index case may serve as a potential indicator of that animal's ability to transmit bTB. This approach allows us to assess genetic variability in infectivity.

To apply the index case approach effectively, clear definitions of both the bTB cases and the infectivity phenotype are essential. The bTB case definitions used in this context align with those established in national genetic evaluation for bTB resistance as described above (Banos et al., 2017).

The concept of the index case refers specifically to those initial animals that test positive and thus trigger the declaration of a bTB breakdown within the herd. Once the breakdown begins, the herd is typically subjected to periodic testing to monitor and control the spread as detailed in Chapter 1. Given the 60-day schedule for bTB testing following the declaration of a breakdown, any new positive cases detected either during the first follow-up test (which occurs approximately 60 days, ± 30 days) only, or within the first or second follow-up test (which occurs approximately 120 days, ± 30 days) were defined as secondary cases. This 30-day detection window was chosen to account for the

disease's latency period as well as to accommodate possible variations in the field testing schedules or delays in data collection. The decision to limit observation to first and second follow-up testing was to ensure that the secondary cases were most likely attributed to the original index case. As time progresses, the likelihood of new infections originating from sources other than the index case increases. This makes it challenging to accurately attribute the source of new infections in subsequent rounds of testing.

Using this approach, two distinct infectivity phenotypes were defined for each index case. The first phenotype, **BINSC (Binary Secondary Cases)**, represents the absence or presence of secondary cases associated with each index case. This phenotype is binary and captures whether an animal is generating secondary cases or not, without quantifying the number of secondary cases it generates. The second phenotype, **NSC (Number of Secondary Cases)**, represents the actual number of secondary cases per index case, which can be zero or greater. The values of both phenotypes depend on the testing periods considered. Specifically, they depend on whether only the first testing period is included, or both the first and second testing periods are considered.

It's important to note that this approach does not assume that every secondary case was directly infected by the index case. It also does not assume that the index case could not have caused new infections later on. Instead, the infectivity phenotype is an estimate of how likely the index case was to generate secondary cases during the initial phases of the outbreak.

2.2.3 Data Filtering & Processing

To prepare the data for analysis, several data edits were applied. Only Holstein-Friesian cows were included in the dataset, considering that the Holstein-Friesian breed is dominating (95% of all dairy cows in the UK (Brotherstone et al., 2010)) and was considered in national genetic evaluation of bTB resistance (Banos et al., 2017). To simplify the infection tracing process and strengthen the association between the index and secondary cases, breakdowns were filtered to include only those initiated by a single index case. Only index cases aged 18 months or older were considered, as this represents the youngest age for milking cows and aligns with the data edit used in the national genetic evaluation of bTB resistance. Additionally, only herds with at least five animals of which at least one was infected, were considered so that herd sizes are large enough to ensure the analysis remains statistically reliable and the threshold aligns with previous studies using similar data (Brotherstone et al., 2010; Banos et al., 2017).

Information from existing variables was also collated into additional simplified ones. For example, the season was determined using the breakdown start date, dividing the year into four seasons: December to February; Winter, March to May; Spring, June to August; Summer, and September to November; Fall. Herd size was calculated at the breakdown start date, and subsequently categorised into four levels (Level 1: 5-68, Level 2: 69-157, Level 3: 158-201, Level 4: greater than 201). These levels were chosen based on the distribution of herd sizes in the dataset (i.e. quartile-based grouping), to ensure reasonably balanced group sizes across levels. Lactation number had nine levels in the

data, but due to low sample sizes beyond the third level, we categorised it into three categories: Level 1 (cows experiencing their first lactation), Level 2 (cows experiencing their second lactation), and Level 3 (cows experiencing their third or subsequent lactations). Furthermore, county information was not directly available in the dataset, but was derived from the CPHH code associated with each herd. This provided only broad location information, and more detailed spatial data (e.g., TB risk or badger density) was not available for inclusion in the models. Additionally, since some herds experienced multiple breakdowns, a unique breakdown ID was generated using the herd ID along with the breakdown start date to ensure each breakdown event was distinct.

The final dataset comprised over 6,668 official bTB breakdowns (index cases), across 4229 unique herds. Pedigree information was trimmed to seven generations to focus on meaningful genetic relationships and to reduce noise, resulting in 70,630 records comprising 2,931 sires.

2.2.4 Non-genetic factors examined in relation with the infectivity phenotype

The influence of potential non-genetic factors on the infectivity phenotype that were available in the bTB dataset of this study, including the age of the index case, herd size, season, breakdown start year, county, and lactation, were investigated using a forward selection approach. The significance of these factors, incorporated as fixed effects in the statistical models outlined below was tested using Wald F-statistics (Butler et al., 2017). This approach was

employed, starting with a simple fixed effects model containing only the intercept. Then, additional fixed effects were added one by one, and at each step, their significance was evaluated using the Wald F-test. Only statistically significant effects were retained in the model, and this process continued until no additional fixed effects contributed significantly to explaining the variation in the infectivity phenotype. This selection process was conducted separately across all models outlined below, and the statistically significant fixed effects in all models were retained to define a consistent linear predictor.

2.2.5 Statistical models

The phenotypes for bTB infectivity described above (i.e., **BINSC** and **NSC**) were examined using linear mixed models and generalised linear mixed models (GLMMs) under a frequentist framework. Five models were explored using NSC as a response variable: (1) a linear mixed model considering the natural logarithmic transformed response variable (2) a linear mixed model considering square root transformed response variable; (3) a Poisson generalised mixed model; (4) a Negative binomial generalised mixed model and (5) a Poisson generalised model considering only index cases with number of secondary cases greater than zero. In addition, (6) a binomial mixed model considering absence or presence of number of secondary cases as the response variable (BINSC) was fitted. In models (1-4) NSC was shifted to NSC+1 to avoid undefined log(0) in the computation and for concordance across the models.

All models were implemented in R using the Asreml R package (Butler et al., 2017).

The following linear predictor was common to all models:

$$\eta_i = ß_0 + ß_{\boldsymbol{X}}\boldsymbol{X}_i + s_i \quad (1)$$

where $ß_0$ is the intercept common to all index cases in this study, $\boldsymbol{X}_i$ is the vector of fixed effects of the index case i and $ß_{\boldsymbol{X}}$ is the corresponding regression coefficient vector, and s_i is the random additive genetic effect of the sire of the index case i. The linear predictor in (1) is presented in general form, and the final form for the linear predictor considering the specific effects considered is presented in equation (5) in the results section.

The joint distribution of random effects in the linear predictor assuming normal distribution with zero mean were as follows:

$$\begin{bmatrix} s \\ e \end{bmatrix} \sim N\left[0, \begin{bmatrix} \boldsymbol{A}\sigma_s^2 & 0 \\ 0 & \boldsymbol{I}\sigma_e^2 \end{bmatrix}\right]$$

where s and e are the vectors of sire genetic and residual effects, respectively, σ_s^2 and σ_e^2 are the corresponding variances; $\boldsymbol{A}$ is the additive relationship matrix, calculated from the pedigree, and $\boldsymbol{I}$ is identity matrix.

2.2.5.1 Generalized Linear Mixed Models

2.2.5.1.1 Poisson Model

A Poisson mixed effect model was fitted to data from all index cases (model 3), and to index cases with $NSC > 0$ (model 5). The conditional probability distribution has the form (Cameron & Trivedi, 2013),

$$P(NSC_i|\lambda_i) = \frac{e^{-\lambda_i}(\lambda_i)^{NSC_i}}{(NSC_i)!} \quad (2)$$

where the mean rate parameter λ_i was related to the linear predictor with the log-link function according to $\lambda_i = e^{\eta_i}$.

2.2.5.1.2 Negative Binomial Model

The conditional probability for the negative binomial distribution (model 4) can be expressed as (Ntzoufras, 2011),

$$P(NSC_i|\mu_i, \theta) = \binom{(NSC_i) + \theta - 1}{NSC_i} (1 - \mu_i)^{NSC_i} {\mu_i}^{\theta}, \quad (3)$$

where θ is a common shape parameter, and the success probability (i.e., the success probability of not generating any secondary cases), μ_i, is related to the linear predictor, η_i, according to the log-link function, which can be given as

$\mu_i = \frac{e^{\eta_i}}{1+e^{\eta_i}}$.

2.2.5.1.3 Binomial Model

For the binomial model, the success probability of getting secondary cases is given as follows (Waddington et al., 1994),

$$P(BINSC_{\ i} = 1|\eta_i) = \frac{e^{\eta_i}}{1+e^{\eta_i}} \quad (4)$$

2.2.5.2 Heritability Estimates

The heritability for all models except for the binomial model was calculated in the underlying scale as $h^2 = \frac{4\sigma_S^2}{\sigma_S^2+\sigma_e^2}$. For the binomial model, $\sigma_e^2 = \frac{\pi^2}{3}$ and the heritability was then calculated as $h^2 = \frac{4\sigma_S^2}{\sigma_S^2+\frac{\pi^2}{3}}$ (Gilmour et al., 2009; Maniatis et al., 2015).

2.2.6 Assessment of Variance Component Estimates

To assess the significance of the sire variance estimates (i.e. whether these are significantly different from zero), we employed the Likelihood Ratio Test (LRT). The LRT is a statistical method for comparing nested models, where a simpler model is tested against a more complex one that includes additional random effects or parameters (Self & Liang, 1987). In our case, the complex model included all the fixed effects and the sire of the index case as a random effect, while the simpler model excluded the sire of the index case. The test statistic was computed as twice the difference in log-likelihoods between the two models, which follows a chi-square distribution under the null hypothesis

that the simpler model is superior. If the result of the LRT test is significant, then the null hypothesis is rejected, indicating that the additional random effect significantly improves the model's fit. We also employed the z-ratio method, which is simpler and computationally efficient. The z-ratio was calculated by dividing the variance component estimate by its standard error. A z-ratio greater than 2 is typically considered evidence that the variance component is significantly different from zero (Nguyen et al., 2007). Both the LRT and z-ratio approaches were implemented directly in ASReml (Butler et al., 2017).

2.3 Results

2.3.1 Descriptive Statistics

The descriptive statistics for the NSC in bTB breakdowns are given in Table 1. The mean NSC is 1.014 in the first testing period and increases to 1.632 when both periods were considered. The skewness of 18.37 and 14.21 for both testing periods, respectively, showed a highly right-skewed distribution, revealing that most breakdowns have either no or very few secondary cases. Additionally, the kurtosis values of 594.90 and 373.35 indicated heavy tails in the distributions.

Table 1: Descriptive Statistics of the NSC associated with each index case.

Statistics	First Testing Period*	First and Second Testing Period*
Min	0.000	0.000
Mean	1.014	1.632
Max	176.000	202.000
SD	4.131	5.422
Skewness	18.370	14.210
Kurtosis	594.900	373.350

*First Testing Period: occurs approximately 60 days, ± 30 days, First and Second Testing Period: occurs approximately 120 days, ± 30 days

When NSC was considered in the first testing period only, the majority of index cases (approximately 73%) had no secondary cases, resulting in a highly skewed distribution (Figure 2a). Considering the index cases with NSC>0, around 40% of these index cases had only one secondary case, while the remaining had multiple secondary cases (Figure 2c). When secondary cases were considered in the first and second testing periods, the distributions remained skewed, but the percentage of breakdowns with zero secondary cases decreased slightly to around 64% for NSC (Figure 2b). For NSC>0, the percentage of index cases that produce multiple secondary cases increased from 60% to around 70% (Figures 2c, 2d). Hence, a few more secondary cases were detected in the later tests, although the general trend of a skewed distribution persists, highlighting that a substantial proportion of breakdowns produce few or no secondary cases, regardless of the testing period. A similar trend was observed for the BINSC phenotype for the first and first two testing periods, where index cases with at least one secondary case increased from 27% to 36% (Figure 2e, 2f).

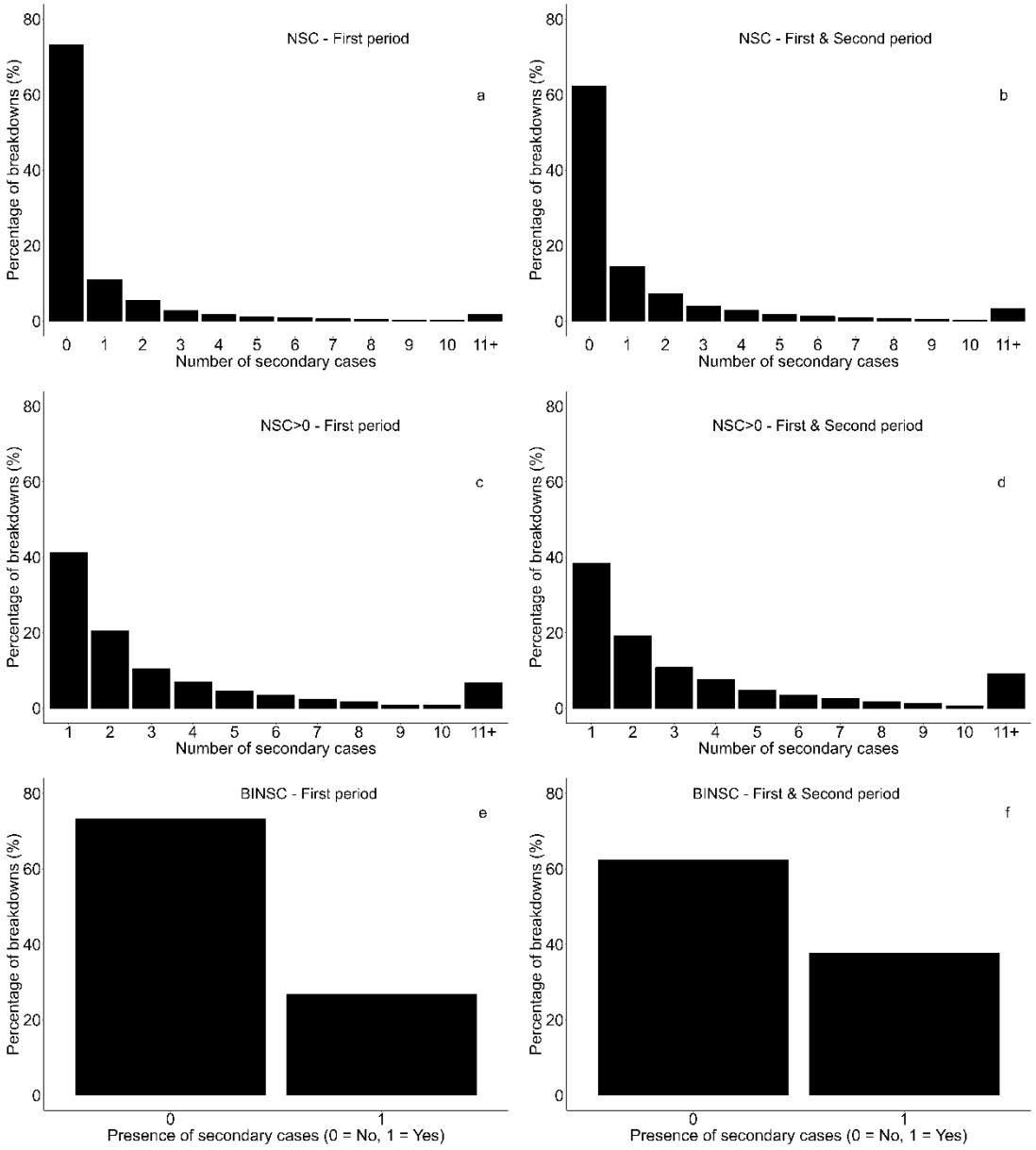

Figure 2: Distributions of secondary cases per breakdown, when secondary cases are defined based on the first or first and second testing periods, respectively, for NSC, and BINSC.

Figure 3 shows the distribution of index cases per sire. The figure revealed that approximately 70% of sires are associated with only one index case. This indicated that the majority of index cases were spread across a large number

of sires, and relatively few sires were associated with multiple index cases. In other words, only about 30% of sires were linked to more than one index case.

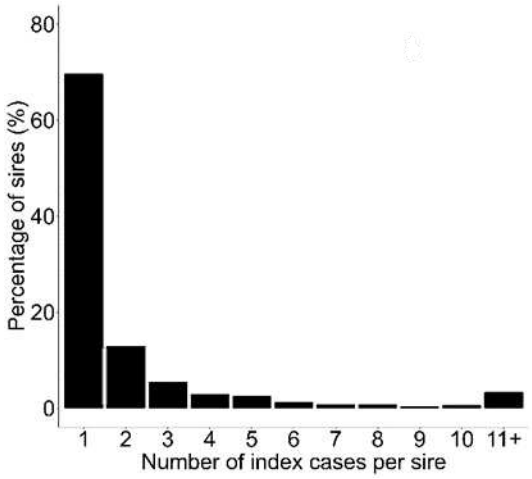

Figure 3: Distributions of index cases per sire

2.3.2 Non-genetic effects and final model

Summary statistics for the potential non-genetic determinants (i.e. the age of the index case, herd size, season, breakdown start year, county, and lactation number) influencing the infectivity phenotype considered in this study are provided in Table 2.

The age of index cases ranged from 550 days to 5,947 days, with an average age of 1,776 days and a standard deviation of 894.34 days, indicating moderate variability around the mean. The age distribution is moderately skewed to the right (skewness = 0.98) and exhibited a kurtosis of 3.75.

Table 2: Summary statistics of the potential non-genetic factors affecting the infectivity phenotype, fitted as fixed effects in the statistical models

Variable	Type	Levels / Statistics
Age (days)	Continuous	Min: 550, Max: 5947, Mean: 1776, SD: 894.34, Skewness: 0.98, Kurtosis: 3.75
Herd size	Categorical	4 levels: 5-68, 69-157, 158-201, >201
County	Categorical	49 levels
Breakdown year	Categorical	22 levels: 2000-2021
Breakdown season	Categorical	4 levels: Spring, Summer, Fall, Winter
Lactation	Categorical	3 levels: 1, 2, ≥3

These factors (and relevant interactions) were fitted as fixed effects in the above models, and Wald F-test was used to assess the fixed effects to be included in the final model. Our analyses revealed several significant fixed effects that account for a significant proportion of the phenotypic variance in the infectivity phenotypes. Specifically, we found that the age of the index case, herd size of the breakdown, county, and the breakdown start year and season interaction were statistically significant predictors (Wald F-statistics p-value <0.05) across all models, leading to following final equation for linear predictor.

$$\eta_{\mathrm{ijkl}} = ß_0 + ß_1 A_i + YS_j + H_k + C_l + s_i \quad (5)$$

where $ß_0$ is the intercept common to all index cases in this study, A_i is the age of the index case i at breakdown onset and $ß_1$ is the corresponding regression coefficient, YS_j is the fixed effect of calendar year by season j of breakdown onset, H_k is the fixed effect of herd size k, C_l is the fixed effect of county l, and s_i is the random additive genetic effect of the sire of the index case i.

2.3.3 Estimates of Variance Components

Table 3 summarizes the estimated variance components, while Table 4 shows the statistical significance of sire variance, evaluated using the LRT and z-ratio.

During the first testing period, both model 1 and model 2 produced very low variance estimates. Similarly, model 6 also yielded small variance estimates. In contrast, model 3 and 5 demonstrated high variance estimates, while the model 4 exhibited moderate variance estimates.

In the combined testing periods, some models, including model 1, 2, and 3, failed to converge, although we tried to solve the convergency problem by increasing the number of iterations. Both model 3 and model 5 maintained variance estimates similar to those observed when analysing data only from the first testing period. However, model 4 showed a reduction in estimates when comparing the results for the first and second period combined to those pertaining only to the first testing period.

Table 4 presents the statistical significance of sire variance across different models. During the first testing period, both model 1 and model 2 failed to detect sire variance significantly different from zero, and model 6 also showed no significant result. In contrast, model 3 and model 5 demonstrated highly significant sire variance, while model 4 also identified significant sire variance with moderate z-ratio.

In the combined testing periods, models (1, 2, and 6) did not converge, preventing significance testing. Both model 3 and model 5 once again showed highly significant sire variance. Although the estimates of the model 4 estimates remained significant according to LRT, its z-ratio was less than two.

Table 3: Variance estimates (SE) for linear and generalised linear mixed models

	First testing period			First and second testing period		
Model	σ_s^2	σ_e^2	h^2	σ_s^2	σ_e^2	h^2
(1) Linear (log)	0.000 (0.002)	0.404 (0.007)	0.001 (0.016)		NC	
(2) Linear (sqrt)	0.000 (0.002)	0.392 (0.007)	0.000 (0.017)		NC	
(3) Poisson	0.380 (0.020)	1.600 (0.030)	0.760 (0.040)	0.400 (0.030)	2.460 (0.050)	0.560 (0.040)
(4) Negative binomial	0.030 (0.010)	0.960 (0.020)	0.100 (0.050)	0.008 (0.009)	1.160 (0.020)	0.020 (0.030)
(5) Poisson (NSC > 0)	0.489 (0.045)	2.096 (0.097)	0.757 (0.072)	0.440 (0.040)	2.910 (0.100)	0.530 (0.040)
(6) Binomial	0.001 (0.020)	1.015 (0.018)	0.002 (0.079)		NC	

NC = not converged, σ_s^2 = sire variance, σ_e^2 = residual variance, h^2 = heritability in the underlying scale

Table 4: LRT (p-value) and z-ratio results for assessing the significance of sire variance

	First Period		First and Second Period	
Model	**LRT (p-value)**	**z-ratio**	**LRT (p-value)**	**z-ratio**
(1) Linear (log)	0.005 (0.473)	0.069	-	-
(2) Linear (sqrt)	0.000 (0.500)	0.003	-	-
(3) Poisson	4338.100 (< 2.2e-16***)	15.948	3266.400 (< 2.2e-16***)	14.658
(4) Negative Binomial	866.530 (< 2.2e-16***)	2.000	209.310 (< 2.2e-16***)	0.830
(5) Poisson (NSC>0)	867.090 (< 2.2e-16***)	10.767	1033.000 (< 2.2e-16***)	11.046
(6) Binomial	0.858 (0.177)	0.031	-	-

2.3.4 Model Fit

To assess how well the models fit with the data, we provided Quantile-Quantile (QQ) plots for each model in Figure 4. Each model demonstrated varying degrees of success in capturing the distribution of the data, with noticeable differences depending on the transformation or statistical model.

The linear models, employing log and sqrt transformations, showed limited success in fitting the NSC data. The log-transformed linear model exhibited significant deviations from the theoretical quantiles, particularly at the extremes, for the first period. While combining the first and second periods improves the fit slightly, the model still struggled to accurately represent the data tails, where extreme values were present. The square root transformation was slightly better, with reduced deviations in the middle quantiles. However, like the log transformation, it failed to address the skewed distribution adequately in both first and combined periods.

Models (3 and 5) also faced challenges fitting the NSC count data. The QQ plots for the model 3, particularly for the first period, revealed substantial deviations at the upper tail, highlighting its inability to capture the distribution of the data. Including only cases with NSC greater than zero (model 5) did not significantly improve the fit, as similar tail deviations were observed for both first and combined periods.

Model 4 provided a better fit for the NSC data. For the first period, the QQ plot demonstrated improved alignment with theoretical quantiles, particularly in the middle range of the distribution, although some deviations persisted at the extremes. When the first and second periods were combined, the fit improved slightly.

The QQ plots for the model 6 revealed significant deviations from the theoretical line for both the first and the combined periods. This poor fit suggested that a binary classification approach failed to capture the distribution of the data.

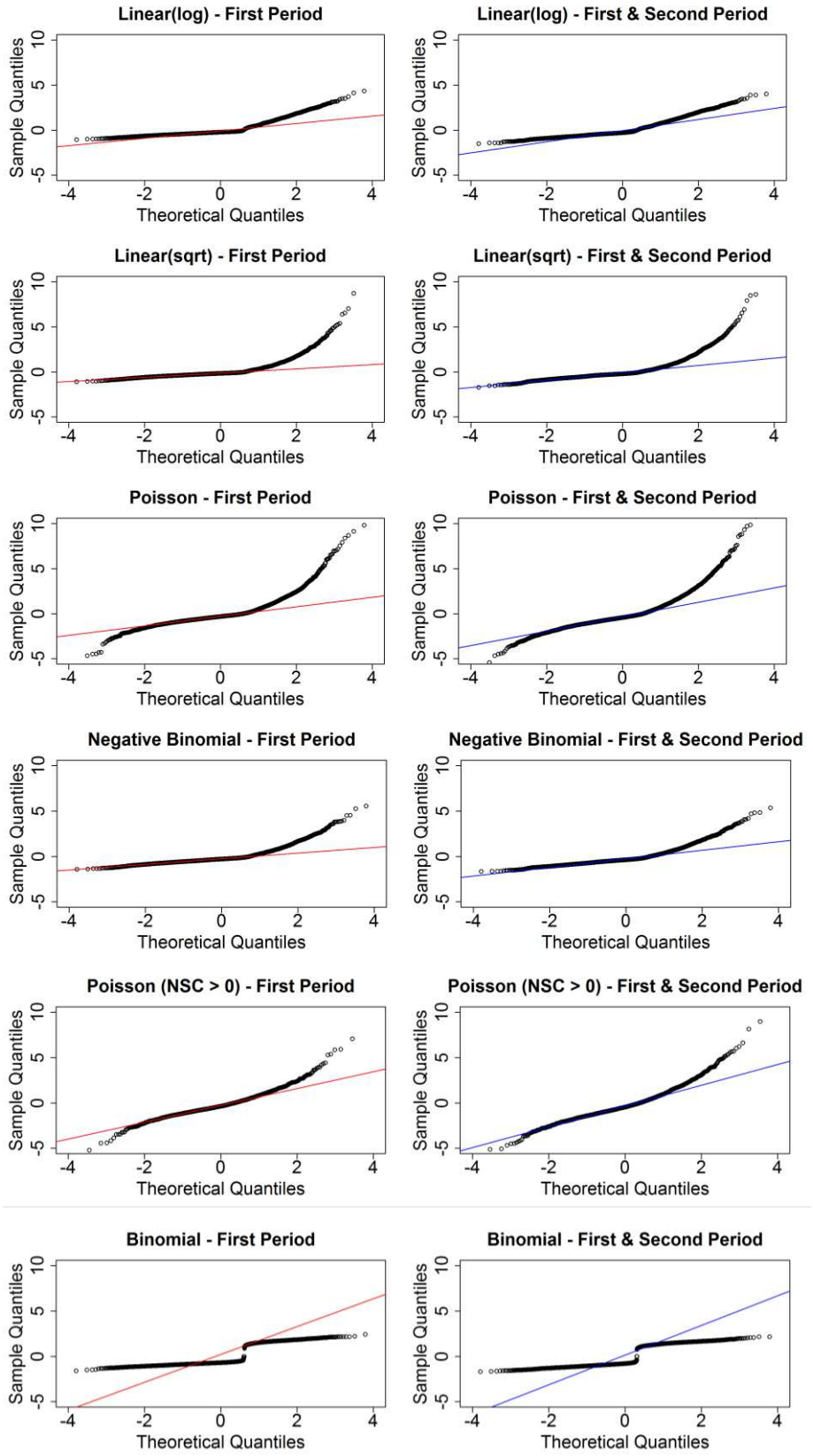

Figure 4: Quantile-Quantile plots of the models to assess the model fit

2.4 Discussion

In this chapter, we investigated candidate bTB infectivity phenotypes and factors potentially influencing bTB infectivity. Introducing the index case approach, we proposed two distinct bTB infectivity phenotypes, namely BINSC (absence or presence of secondary cases) and NSC (number of secondary cases) attributed to the index case. We utilised the national evaluation GB bTB dataset, employing six alternative models.

2.4.1 Non-genetic factors

Using forward selection, we identified common non-genetic predictors that explained a significant proportion of the phenotypic variation of the infectivity phenotypes considered in this chapter. Some previous studies (Cadmus et al., 2010; Brooks-Pollock et al., 2013; Broughan et al., 2016) have shown that the prevalence of bTB infection increased with age, and cattle aged 12 to 36 months appeared to be the most vulnerable to infection. Considering this, we assessed whether age has any effect on the infectivity phenotypes, too. Our results revealed that age was one of the key predictors of the infectivity phenotypes. This may be attributed to the fact that the contact duration and exposure to infection increases with age (Skuce et al., 2011), which may lead to a higher probability of getting the disease and subsequent transmission.

Herd size was also found to have a significant impact on the bTB infectivity phenotypes. Larger herds inherently have more animals, leading to a higher

number of potential contacts between individuals. This increased interaction rate may facilitate the spread of disease, as infected cattle have more opportunities to transmit the pathogen (Conlan et al., 2012; Broughan et al., 2016). We categorized herd size into groups rather than considering it as a continuous covariate to account for the challenges of defining a "true" herd size, which may change during the breakdown due to deaths or culling during the study period.

Geographical variation, captured through the inclusion of county as a fixed effect in our analyses, was found to be statistically significant, suggesting that environmental conditions and regional farming practices may also play a critical role in disease transmission. For example, different regions exhibit variations in climate, soil type, and vegetation, which may affect the survival and transmission of *M. bovis* in the environment (Broughan et al., 2016). Also, regional differences in farming practices, including herd management, biosecurity measures, and grazing strategies, may influence disease transmission dynamics (White & Benhin, 2004).

Temporal factors, such as the breakdown start year and season, were also found to be statistically significant in our analyses of the infectivity phenotypes. Seasonal variations are likely to play a role in the spread of bTB, as diverse factors such as temperature and moisture levels, which fluctuate with the seasons, can affect the survival and transmission rates of *M. bovis* (Broughan et al., 2016), as mentioned earlier.

In our analyses, lactation number, tested as a potential fixed effect for bTB infectivity, did not emerge as statistically significant. This result may be attributed to the inclusion of age as a covariate in our models, considering the potential correlation between lactation number and age of the index case. By incorporating age, any potential influence of lactation number on bTB infectivity might have been confounded or masked.

2.4.2 Genetic variance Estimates

Our results highlighted that genetic variance was significantly different from zero in certain models, particularly those assuming Poisson and Negative Binomial distributions. Both model 3 and 5 consistently identified significant sire variance across both the first and combined periods, with high LRT values and z-ratios. The Poisson models yielded high genetic variance estimates, with model 5 (NSC>0) slightly higher than model 3. The inclusion of only positive NSC cases in model 5 likely reduced the influence of zeros in the data, improving its ability to detect and quantify genetic variance. However, this approach could potentially exclude valuable information about the absence of secondary cases, which might also have a genetic contribution. The Negative binomial approach (model 4) also detected significant sire variance, with moderate z-ratios. In contrast, the models with log and sqrt transformations (models 1 and 2) and the Binomial (model 6) failed to detect significant sire variance.

While some of the results highlight the potential for genetic variation in infectivity, it is important to note that in this study, approximately 70% of sires were associated with only a single index case. This means that only around 30% of sires had multiple records, which are essential for accurate estimation of genetic parameters. When a sire has only one associated index case, it becomes difficult to separate true genetic effects from individual-level noise. As a result, the low number of repeated observations per sire likely compromised the precision of our variance estimates, and may partially explain why some models failed to detect statistically significant sire variance. Moreover, the structure of genetic relatedness within the dataset may substantially influence the estimation of genetic parameters, either increasing or reducing genetic variance. Therefore, the low proportion of replicated sires might contribute to the uncertainty and variation observed in the genetic estimates across models.

2.4.3 Testing Periods

The results indicated that models using log and sqrt transformations failed to estimate significant sire variance in the first testing period and failed to converge when the testing periods were expanded, likely due to the violation of the normality assumption. Similarly, the binomial approach (model 6), which oversimplifies the variability of NSC counts through binary classification, did not yield significant sire variance estimates in the first testing period and failed to converge in the combined testing period.

The Poisson models (models 3 and 5) produced very high sire variance estimates, particularly during the first testing period, but did not show improvement in the combined period. The Negative Binomial model (model 4) showed a decline in sire variance when moving from the first period to the combined period and sire variance was not statistically significant in the combined period. This may suggest that genetic variance might be more detectable in early stages of breakdown, considering that the likelihood of newly infected animals being infected by index cases may decrease in further test periods and might obscure the genetic signal.

2.4.4 Infectivity Phenotypes

The choice of phenotype also influenced model performance and variance estimates. Models designed for count data (Poisson and Negative Binomial) were better suited for the NSC phenotype, as they align with the discrete nature of the data. Transformations of NSC (log and sqrt) were insufficient to address the normality assumption, leading to poor variance estimates (O'Hara & Kotze, 2010) and a lack of convergence in the combined period. A binary phenotype such as the one used in the Binomial model fails to capture differences in bTB infectivity based on different numbers of secondary cases. The results suggest that the NSC phenotype, treated as count data, provides better insights for bTB infectivity compared to the transformed and binary alternatives.

2.4.5 Model Fit

Discrete response variables may include a high proportion of zero observations, which may result in deviations from normality. To address this, transformations are often applied to the response variables to enhance model fit by improving the linearity between the response and predictors (O'Hara & Kotze, 2010). We followed a similar approach in the models with log and sqrt transformations. The results for model fit highlighted that transformations (log and sqrt) failed to address the normality assumption, likely due to the highly skewed distribution of the NSC. Similarly, the binomial model was also insufficient in capturing the particular distribution of the data.

The Poisson models struggled to fully capture the particularities of the NSC distribution although they produced significant sire variance estimates. This might be due to the assumption of the Poisson distribution that the mean and variance are equal (Cameron & Trivedi, 2013), which may not be the case in our NSC phenotype, considering that 70% of the NSC was zero. On the other hand, the Negative Binomial (model 4) demonstrated a better fit, which might be attributed to not assuming equal mean and variance as Poisson, resulting in better accounting for potential over-dispersion in the data, although this needs to be further investigated. However, even with the Negative Binomial approach, challenges remain in capturing the extreme values in the distribution.

While the Poisson and Negative Binomial models produced significant sire variance estimates and demonstrated relatively better model fit, they were still insufficient to fully capture the bTB infectivity. In the case of the Poisson

models, the heritability estimates were notably higher than the range typically reported in the literature for bTB susceptibility and resistance, which generally falls between 0.06 and 0.18 (Banos, 2023). For the Negative Binomial model, the heritability estimates aligned more closely, however, the moderate z-ratio raises concerns about their statistical significance. It is also important to note that these estimates were presented on the underlying scale, making direct comparisons challenging. Therefore, while these models offered some insights, they may not be sufficient to draw conclusions about genetic variance in bTB infectivity. This highlights the need for further refinements or the exploration of more advanced methodologies.

2.5 Conclusions

The results obtained in this chapter offer the first evidence of potential genetic variation underlying bTB infectivity, defined as the number of secondary cases at the early stages of a breakdown that may be attributed to the initial index case, suggesting that genetic factors may play a role in determining the extent to which individual animals contribute to disease transmission within herd. These are first, exploratory data analyses linked with certain limitations and challenges discussed above. The next chapter will address these limitations by testing for overdispersion and zero inflation, given the highly skewed distribution with 70% zero secondary cases. Additionally, advanced statistical models will be explored to improve model fit and provide deeper insights to better account for factors influencing bTB infectivity and transmission dynamics.

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Chapter 3

Detection of genetic variability in dairy cattle infectivity for bovine tuberculosis

3.1 Preface for the Chapter

This chapter consists of the article entitled "Detection of genetic variability in dairy cattle infectivity for bovine tuberculosis", which has been published in the Journal of Dairy Science. This chapter addresses the second objective of the thesis to detect genetic variability in the novel infectivity phenotype and estimate its variance components through the use of different statistical models. Additional information and analyses related to this chapter are provided in Appendix A and Appendix B.

Full citation of the published paper:

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3.2 Manuscript

J. Dairy Sci. 108:3835–3849
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Detection of genetic variability in dairy cattle infectivity for bovine tuberculosis

D. Madenci,[1] E. Sánchez-Molano,[1] M. Winters,[2] A. Mitchell,[3] M. P. Coffey,[4] J. D. Hadfield,[5] J. A. Woolliams,[1] G. Banos,[4]* and A. Doeschl-Wilson[1]*†

[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian EH25 9RG, United Kingdom
[2]Agriculture and Horticulture Development Board (Dairy), Stoneleigh Park, Kenilworth, Warwickshire CV8 2TL, United Kingdom
[3]Animal and Plant Health Agency, Surrey KT15 3NB, United Kingdom
[4]Scotland's Rural College, Easter Bush, Midlothian EH25 9RG, United Kingdom
[5]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3FL, United Kingdom

ABSTRACT

This study investigated the genetics of bovine tuberculosis (bTB) infectivity in Holstein-Friesian dairy cows using British national data. The analyses included cows with recorded sires from herds affected by bTB outbreaks between 2000 and 2022. Animals were considered bTB positive if they reacted positively to the skin test, had positive postmortem findings, or both. We introduced the "index case approach," based on the assumption that once the initial positively tested animals (index cases) are detected in a herd, subsequent infections (secondary cases) in the early stages of the breakdown are likely to be attributed to these animals. Genetic analysis of the number of secondary cases (NrSC) associated with a given index case was used to establish evidence of genetic variability in bTB infectivity of cattle, and derive EBV for infectivity for the sires of the index cases. Data were analyzed by employing Markov chain Monte Carlo techniques to fit generalized linear mixed models with either Poisson, zero-inflated Poisson (ZIP), hurdle Poisson, or geometric distributions. All 4 models demonstrated presence of genetic variance in cattle infectivity, with the strongest evidence provided by the ZIP and hurdle Poisson models. The hurdle Poisson model offered the most accurate and least biased predictions. Sire infectivity EBV from the Poisson, ZIP, and geometric models showed strong concordance, with pairwise correlations of 0.90 or higher. In contrast, correlations between EBV from the hurdle Poisson model and the other models ranged from 0.36 to 0.39. The association of the sire infectivity EBV with the average observed NrSC per sire and the proportion of infectious index case daughters per sire was generally moderate with correlations between 44% and 47% and 65% to 69%, respectively. Agreement among models for identifying the genetically most infectious sires was also reasonable, with 151 out of 285 sires appearing in the top 10% across models, and 122 (42.8%) also aligning with the top 10% based on observed average NrSC. Results provide novel evidence for exploitable genetic variance in bTB infectivity allowing the derivation of meaningful EBV. Based on the estimated posterior mean genetic variances obtained, reduction in infectivity by 1 genetic SD would result in a 32% to 44% decrease in the expected NrSC per index case. Further research is warranted to refine the phenotypic definition of infectivity and assess correlation with other dairy traits.

Key words: genetic analysis, bovine tuberculosis, infectivity, dairy cattle

INTRODUCTION

Bovine tuberculosis (**bTB**), an infectious disease caused by the bacterium *Mycobacterium bovis*, continues to pose a significant threat to animal and human health worldwide. The bacteria are primarily transmitted via respiratory droplets through direct contact with infected individuals (Domingo et al., 2014), extending to various species of mammals, thereby complicating eradication efforts. To curb its spread, comprehensive control strategies are crucial, involving accurate and timely diagnostics, stringent biosecurity measures, and targeted interventions, such as culling, vaccination, or breeding (Byrne et al., 2019).

In the United Kingdom, bTB persists within the cattle population, particularly in south western England, Wales, and Northern Ireland, where the majority of bTB cases were reported (Abernethy et al., 2013; Allen et al., 2018; Mitchell et al., 2022). The disease causes a substantial financial strain on both farmers and taxpayers, encompassing costs over £150 million (equivalent to $185 mil-

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*These authors contributed equally to this work.
†Corresponding author: andrea.wilson@roslin.ed.ac.uk

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

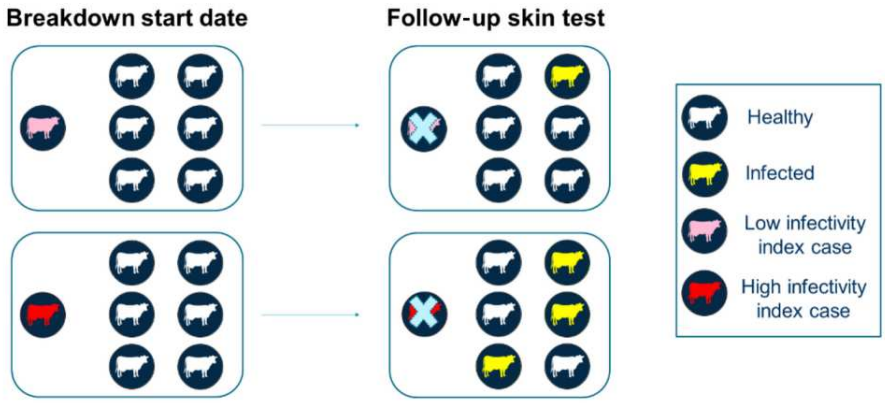

Figure 1. Index case approach: defining the infectivity of index cases triggering a breakdown based on the number of infected secondary cases identified in the follow-up skin test.

lion) annually for bTB control and eradication programs that include not only direct losses from culling animals diagnosed as bTB positive but also the implementation of routine and targeted surveillance schemes, movement restrictions, biosecurity measures, and trade disruptions on infected herds (Godfray et al., 2013; Mitchell et al., 2022). Control efforts extend also to the development of vaccines for cattle and wildlife reservoirs, particularly Eurasian badgers, which are the primary wildlife reservoir in the United Kingdom. Vaccination of badgers with the Bacillus Calmette-Guérin vaccine has recently been introduced in the United Kingdom, and field trials for cattle are currently underway. In addition, targeted badger culling has been trialed. However, studies in the United Kingdom have reported ambiguous results (Redpath et al., 2023; Birch et al., 2024).

The UK government's comprehensive bTB eradication strategy involves a stringent surveillance and culling regimen in cattle herds (TbHub, 2023). Surveillance is done by routine testing of herds at a frequency between 6 mo to 4 yr, depending on the herd location within designated bTB risk areas (namely, "low risk" areas, requiring tests every 4 yr, "edge" areas requiring tests every 6 or 12 mo, and "high risk" areas requiring tests every 6 mo; Salvador et al., 2018; APHA, 2023). The single intradermal comparative cervical test (**SICCT**), commonly referred to as "the skin test," is currently the main in vivo test routinely used to identify infected cattle. It involves injection of 2 types of tuberculin (avian and bovine) into the skin and measuring the relative size of reaction to both types of injections. The animal is declared a reactor (positive result) when its reaction to the bovine tuberculin exceeds the avian tuberculin by more than 4 mm (2 mm in high-risk areas). If the reaction at the bovine tuberculin site is slightly larger than the reaction at the avian site but not large enough to be classified as a reactor, the test is declared as inconclusive and requires repetition after a period of 2 mo. If within a herd, at least 1 cow has either 1 positive or 2 consecutive inconclusive test results, this triggers the declaration of a breakdown. This leads to significant implications for the herd: The herd is subjected to immediate movement restrictions, and its Official bTB free (**OTF**) status is withdrawn. Single intradermal comparative cervical testing of every cow is performed every 60 d. Positive animals and those with 2 consecutive inconclusive test results are immediately sent to slaughter, where they are tested for bTB lesions (de la Rua-Domenech et al., 2006). This comprehensive testing persists until all animals within the herd achieve negative test results in 2 consecutive tests, after which the herd receives the OTF status and restrictions are lifted. Additionally, the bTB surveillance scheme is informed by routine postmortem inspections of all cattle carcasses at slaughterhouses. During these inspections, any cattle showing visible lesions indicative of bTB are identified, and lesion samples are taken for laboratory confirmation. Positive lesion scores from these inspections are used as an additional criterion for bTB detection, complementing the SICCT results to ensure a thorough assessment of the herd's bTB status.

Although successful implementation of bTB control and eradication programs in Scotland resulted in the attainment of the OTF status in 2009, other UK areas have reported more moderate outcomes. This can be partly attributed to the fact that although the routinely used SICCT has high specificity (99.98%), its sensitivity is notably lower, within the range of 50% to 80% (Nuñez-Garcia et al., 2018). This compromises its ability to de-

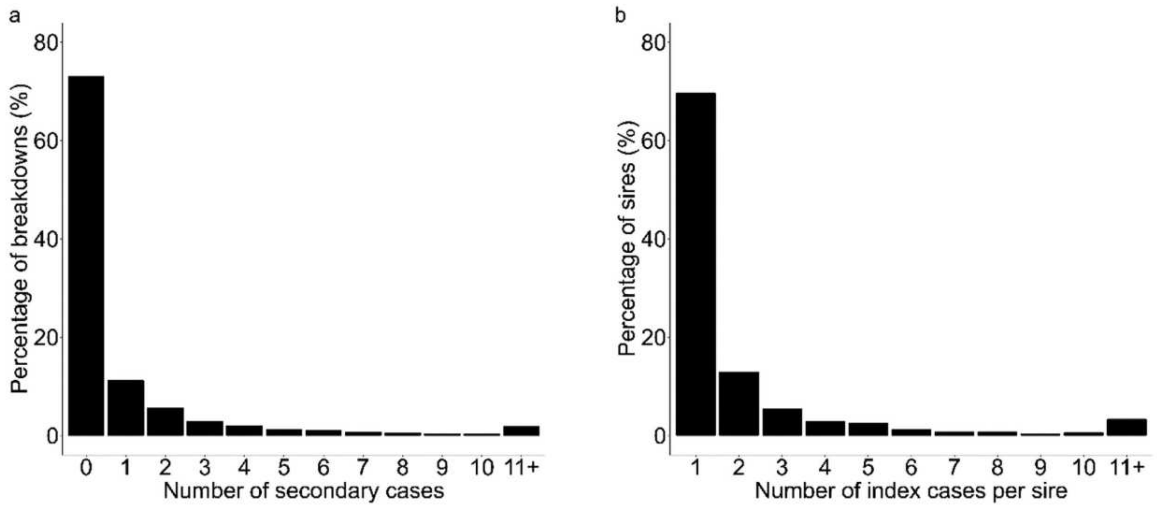

Figure 2. Distributions of secondary cases per breakdown (a) and index cases per sire (b).

tect all truly infected animals in the herd. Nevertheless, a target has been set to achieve OTF status across the whole of Great Britain by 2041 or earlier. However, the enduring challenges in eradicating bTB emphasize the importance of continued exploration into supplementary disease control interventions that can effectively complement existing strategies (Allen et al., 2010).

Breeding cattle for improved bTB resistance has been largely recognized as a promising long-term complementary bTB control strategy (DEFRA, 2018), as evidence for substantial genetic variation in host resistance continued to increase (Allen et al., 2010; Brotherstone et al., 2010). In particular, large-scale genetic studies were facilitated by combining the extensive national databases of bTB individual animal records with the data routinely collected for national genetic evaluations. The combination of the SICCT results, postmortem bTB lesions, and *M. bovis* culture test results allowed for the characterization of the health status of each animal at the corresponding test or slaughter date, effectively enabling the development of a resistance phenotype, which is the propensity to not become bTB test positive (Banos et al., 2017). Previous studies on the genetic variation underpinning bTB resistance have reported heritability estimates between 0.07 and 0.34, making it feasible to carry out genetic selection for increased bTB resistance (Bermingham et al., 2009; Brotherstone et al., 2010; Raphaka et al., 2017). Although genomic regions and candidate genes underlying bTB resistance have been reported, genomic studies seem to confirm that bTB resistance has a complex polygenic architecture (Richardson et al., 2016; González-Ruiz et al., 2019; Ring et al., 2019).

Based on the cumulative evidence from previous bTB studies, the "TB Advantage" selection index was introduced to the UK dairy industry in 2016, enabling farmers to select bulls with enhanced genetic bTB resistance (BCBC, 2016). Selective breeding based on this index is expected to accelerate the rate of decline in bTB incidence and severity already achieved by eradication strategies. Simulation studies mimicking the UK bTB scenario predicted that, after ~5 generations of rigorous selection of sires with the highest genetic merit for bTB resistance, the likelihood of a breakdown occurring would be reduced by 50%. Similarly, breakdown duration could be reduced by 5% to 10% and severity (i.e., number of bTB positive animals) could be reduced by 11% to 17% following just 1 generation of selection (Raphaka et al., 2018; Banos, 2023). Although difficult to evaluate, the voluntary uptake of the TB Advantage index may lead to a lower selection intensity and moderate realized progress.

Although these simulation and empirical studies provide supportive evidence that genetic selection for bTB resistance is an effective complementary strategy, they also suggest that this alone may not be sufficiently effective to achieve vast reductions in bTB breakdowns within the next decade, and additional breeding goals must be sought to accelerate this (Tsairidou et al., 2018; Banos, 2023). A potential trait to be considered is host infectivity, defined as the ability of an animal to trans-

mit the infection to other animals (Brooks-Pollock et al., 2015; DEFRA, 2018; Doeschl-Wilson et al., 2021). A recent study compiled evidence for substantial phenotypic variation in cattle bTB infectivity (Tsairidou et al., 2018). As such, if genetic variation for bTB infectivity in cattle exists and can be estimated, selecting for infectivity in addition to resistance could substantially accelerate the rate of decline in bTB risk and prevalence. However, the degree of improvement would depend on factors such as the intensity of selection, the heritability of infectivity, prediction accuracy, and the genetic relationship between infectivity and resistance (Tsairidou et al., 2018, 2019; Banos, 2023).

However, infectivity cannot be measured directly and is difficult to disentangle from susceptibility using conventional genetic models applied to disease data (Lipschutz-Powell et al., 2012; Bijma et al., 2022). Numerous methods have been proposed to estimate the genetic basis of host infectivity using epidemiological data. Although one approach is to consider infectivity as an indirect genetic effect within a generalized linear mixed model framework (Lipschutz-Powell et al., 2012; Anche et al., 2014; Biemans et al., 2017), to make these genetic models consistent with epidemiological theory, various simplifying assumptions and approximations need to be made, introducing potential bias in the estimates. Genetic-epidemiological models have also been embedded into hierarchical Bayesian inference methods to estimate genetic parameters related to infectivity without the need for numerical approximations (Anacleto et al., 2015; Doeschl-Wilson et al., 2018; Pooley et al., 2025). Application of these methods to data from experimental disease challenge studies has provided encouraging evidence that genetic variation in infectivity is estimable and of similar magnitude as genetic variation in resistance (Prentice et al., 2022), but their upscaling to the complex and large national bTB field and genetic data, although feasible (Tsairidou et al., 2018), would require extensive adaptations and validation.

As a first step toward breeding for reduced bTB infectivity of cattle, the present study evaluates a proxy bTB infectivity phenotype in dairy cows and investigates the presence of genetic variability among sires with regards to daughter infectivity.

MATERIALS AND METHODS

Data

This study used the data assembled for the national genetic evaluation of bTB resistance in dairy cattle (Banos et al., 2017; AHDB, 2018). Here we focus on bTB test records in Great Britain during the period between 2000 and 2022. In addition to the bTB test results for each cow at each test, there is also information about the age at test, lactation number, breed, county of herd location, breakdown start and end dates, and the herd size at the start of the breakdown. Data also include national pedigree information and sire EBV for bTB resistance in a breakdown.

Defining the Infectivity Phenotype Using the Index Case Approach

Overall Concept. The index case approach is based on the assumption that once the initial positively tested animals (index cases) are detected in a herd, subsequent infections in the early stages of the breakdown (secondary cases) are likely to be infected by these animals. Figure 1 illustrates the key concept of the index case approach by showing 2 bTB breakdowns initiated by 1 index case each. If these 2 animals differ in their infectivity (high/low), the bTB breakdown initiated by the animal with high infectivity is expected to result, on average, in more secondary cases than the bTB breakdown initiated by the animal with low infectivity. Although this pattern could be attributed to the stochastic nature of disease transmission, to other external factors, or both, observing it across genetically related index cases would suggest that the number of secondary cases associated with an index case is partly genetically determined. Therefore, the number of secondary cases associated with an index case in a bTB breakdown is a potential indicator of cattle infectivity for bTB to assess genetic variability in this trait.

Defining bTB Cases and Infectivity Phenotype. The proposed index case approach requires the definition of bTB cases and identification of the index case(s) and secondary case(s) for each breakdown. For this purpose, we adopted the bTB case definition used in the national genetic evaluation for bTB resistance (Banos et al., 2017), based on both skin test and postmortem examination. Specifically, any animal with a positive response to the skin test, positive postmortem results, or both was defined as a bTB case.

We define the index cases as the positive detected cases that initiate the declared start of the breakdown. Given the 60 d bTB testing schedule after a breakdown is declared, secondary cases were then defined as the positive cases identified at the follow-up test at ~60 d (±30 d) after the detection of the index case. A 30-d detection window was chosen to encompass the disease latency period and to accommodate small changes in the field testing schedules and delays in the data collection. The main reason for truncating the observation period to the first follow-up test is that the likelihood of new infections being caused by nonindex cases increases over subsequent test periods.

In line with the previous definitions, we defined the infectivity phenotype for each bTB index case as the number of secondary cases (**NrSC**) detected in that breakdown. We do not assume that every secondary case was necessarily infected by the index case, nor that other cases at a later time were not infected by the index case. The infectivity phenotype presented in this study is thus a proxy to assess the potential of the index case to generate a given number of secondary cases in a breakdown. As such, it should not be interpreted as a strict measure of causality.

Data Filtering and Processing

Only breakdowns with a minimum herd size of 5 animals and with Holstein-Friesian cows were kept in the analysis. Only index cases aged 18 mo or older were considered, as this represents the youngest age for milking cows and aligns with the data edit used in the national genetic evaluation of bTB resistance. Furthermore, to strengthen the links between secondary cases and index cases, only breakdowns initiated by a single index case were considered, resulting in 6,549 breakdowns/index cases. To achieve this, we carefully examined each breakdown individually and calculated the number of index cases in every breakdown. In cases where multiple animals could potentially serve as the index case (e.g., if more than 1 animal was defined as positive detected cases that initiate the declared start of the breakdown), we discarded these breakdowns in our analyses, that is, only kept breakdowns with a single index case. The final dataset contained bTB test results for 1,131,759 animals in 4,143 affected breakdown herds. Demographic information considered in the statistical models were county, breakdown start year and season, and herd size. For each index case, the lactation number and the age at the first positive bTB test result (or second inconclusive test) were also recorded. In addition, to avoid potential confounding between the infectivity of the index case and the resistance of the herd members, the average sire EBV resistance of all herd members was calculated for each breakdown. Finally, pedigree information for all index cases was trimmed to 7 generations (70,630 records) comprising 2,854 sires.

Statistical Models

Preliminary Data Inspection and Tests for Overdispersion and Zero Inflation. Preliminary visual inspections of the distribution of secondary cases associated with each index case suggested that the infectivity phenotype used in this study followed an overdispersed, zero-inflated distribution (Figure 2a). Therefore, statistical tests for overdispersion (Cameron and Trivedi, 1990) and zero inflation (Lüdecke et al., 2020) were performed. Both tests are based on a generalized linear model (**GLM**) for Poisson distributed data but are also valid for other distributions (Cameron and Trivedi, 1990). Specifically, the methodology proposed by Cameron and Trivedi (1990) assesses the mean-variance relationship $var(X) = \mu + c \times f(\mu)$, where X represents the variable under study (in our case, the number of secondary cases attributed to the index case), μ denotes the mean of X under the GLM model, f refers to a function typically parameterized so that a value of $c < 0$ indicates underdispersion, whereas $c > 0$ indicates overdispersion. Zero inflation was tested independently by calculating the ratio of observed count of zeros in the data over the model-predicted count of zeros, where a ratio above 1 indicates zero inflation. These tests were implemented in R (version 4.2.0; https://cran.r-project.org/bin/windows/base/old/4.2.0/) using the packages "AER" (for overdispersion) and "performance" (for zero inflation).

In addition, zero inflation was also assessed using posterior predictive checks (Supplemental Figure S1, see Notes) after implementing the full Poisson model as outlined further in the text, without random sire effect, and comparing the posterior distribution of predicted number of zero secondary cases with the observed number. Unlike the separate tests for overdispersion and zero inflation, the posterior predictive checks simultaneously account for both overdispersion and zero inflation.

Generalized Linear Mixed Models. Based on the outcomes of preliminary data inspections, we explored 4 types of generalized linear mixed models (**GLMM**) known for their ability to accommodate both overdispersion and zero inflation. These were the Poisson model (Majo and van Soest, 2011), the zero-inflated Poisson (**ZIP**) model (Lambert, 1992), the hurdle Poisson model (Mullahy, 1986), and the geometric model (van Oppen et al., 2023), a special case of the negative binomial model.

Common Linear Predictor

All 4 models mentioned previously assume that the observed scale for the phenotype of interest (i.e., infectivity) arises from an underlying normally distributed variable described by a common linear predictor.

In this study, the common linear predictor for infectivity of an index case i was

$$\eta_{ijkl} = \beta_0 + \beta_1 A_1 + \beta_2 R_i + YS_j + H_k + C_l + s_i + e_{ijkl}, \quad [1]$$

where β_0 is the intercept common to all index cases in this study, A_1 is the age of the index case i at breakdown onset (ranging from 550 to 5,880 d) and β_1 is the corresponding regression coefficient, R_i is the average estimated genetic merit for bTB resistance of all herd members of index

case i at the onset of the breakdown (estimated from the available EBV) and β_2 is the corresponding regression coefficient, YS_j is the fixed effect of calendar year by season j of breakdown onset (22 × 4 levels), H_k is the fixed effect of herd size k (which varied between 5 and almost 5,000 animals, and was categorized as a factor with 4 levels based on quartiles), C_l is the fixed effect of county l (factor, 49 levels), and s_i is the random additive genetic effect of the sire of the index case i. e_{ijkl} is an observation-level random effect that accounts for any overdispersion (Hinde, 1982).

The final Model [1] included only nongenetic predictors that were found statistically significant in at least one of the models. The average genetic merit for bTB resistance in each breakdown, though not statistically significant (i.e., did not improve the model fit), was included to avoid potential confounding with index case infectivity.

The joint distribution of the random sire and observation-level effects for the normally distributed underlying variable are as follows:

$$\begin{bmatrix}\mathbf{s}\\ \mathbf{e}\end{bmatrix} \sim MVN\left\{0, \begin{bmatrix}\mathbf{A}\sigma_s^2 & 0\\ 0 & \mathbf{I}\sigma_e^2\end{bmatrix}\right\},$$

where $\mathbf{s}$ and $\mathbf{e}$ are the vectors of sire and observation-level effects, respectively, σ_s^2 and σ_e^2 are the corresponding sire and observation-level variances; $\mathbf{A}$ is the pedigree-based additive relationship matrix, and $\mathbf{I}$ is identity matrix.

Poisson and ZIP Models

For the Poisson distribution, the mean rate of occurrence of secondary cases, λ_{ijkl} is related to the linear predictor by the log-link function $\lambda_{ijkl} = \exp(\eta_{ijkl})$, with j, k, l as defined in Model [1]. The conditional probability of a given number of secondary cases $NrSC_i$ associated with index case i for the Poisson model is then given by

$$p\left(NrSC_i;\lambda_{ijkl}\right) = \frac{\left(\lambda_{ijkl}\right)^{NrSC_i}}{NrSC_i!}e^{-\lambda_{ijkl}}. \quad [2]$$

The ZIP model consists of 2 parts: a Poisson count model for the number of secondary cases and a logit model for predicting extra zeros. More specifically, the zeros observed in the data are thought to arise from 2 different processes: one attributed to the Poisson process of generating count data (i.e., secondary cases), including zeros, and a separate process of zero inflation. The 2 corresponding latent parameters of the ZIP distribution, (π,λ), are linked (using the logit-link and log-link functions, respectively) to 2 different linear predictors represented by Equation [1] with different values for the regression coefficients β_j and fixed and random effects.

The conditional probability of $NrSC_i$ in a ZIP model is then defined as

$$f\left(NrSC_i;\ \lambda_{ijkl},\pi_{ijkl}\right) = \begin{cases}\pi_{ijkl} + \left(1-\pi_{ijkl}\right)p\left(NrSC_i = 0;\ \lambda_{ijkl}\right) \text{if } NrSC_i = 0,\\ \left(1-\pi_{ijkl}\right)p\left(NrSC_i;\ \lambda_{ijkl}\right) \text{if } NrSC_i > 0\end{cases}, \quad [3]$$

where $p\left(NrSC_i;\lambda_{ijkl}\right)$ is the Poisson density function given in Equation [2] and π_{ijkl}, with $0 \le \pi_{ijkl} \le 1$, refers to the probability of additional zero $NrSC_i$ not already accounted for by the Poisson process.

Hurdle Poisson Model

Similar to ZIP, the hurdle Poisson model is a 2-component mixture model, but the latter consists of a zero mass and the positive observations component following a truncated Poisson distribution. In this model, the infectivity phenotype is also associated with a pair of latent variables denoted as (π,λ), each linked to corresponding linear predictors given by Equation [1]. For the hurdle model, the first latent variable (π) represents the probability, on the logit scale, of whether the response variable takes a zero value or not, and the second latent variable (λ) refers to the mean rate parameter of a zero-truncated Poisson distribution.

The conditional probability for observing $NrSC_i$ a zero-truncated Poisson distribution in a hurdle Poisson model is then defined as (Hadfield, 2014)

$$f\left(NrSC_i;\ \lambda_{ijkl},\pi_{ijkl}\right) = \begin{cases}\pi_{ijkl}\ if\ NrSC_i = 0,\\ \left(1-\pi_{ijkl}\right)\dfrac{p\left(NrSC_i;\ \lambda_{ijkl}\right)}{1-p\left(NrSC_i = 0;\ \lambda_{ijkl}\right)} \text{if } NrSC_i > 0\end{cases}, \quad [4]$$

where $p(NrSC_i;\ \lambda_{ijkl})$ is the Poisson density function given in Equation [2] and $0 \le \pi_{ijkl} \le 1$.

Geometric Model

The geometric distribution is equivalent to the negative binomial distribution with a shape parameter (α) set to 1. The corresponding conditional probability function for $NrSC_i$ (van Oppen et al., 2023) is expressed as

Table 1. Posterior estimates for the variance components[1]

Variance[2]	Estimate	Model			
		Poisson	ZIP	Hurdle	Geometric
σ_s^2	Mean	0.0293	0.0197	0.0328	0.0288
	Median	0.0164	0.0085	0.0196	0.0163
	95% HPD[3]	$<10^{-5}$, 0.1071	0.0002, 0.0715	0.0004, 0.1021	$<10^{-5}$, 0.0979
	10th percentile[4]	0.0007	0.0014	0.0030	0.0007
σ_e^2	Mean	4.202	3.884	1.2990	3.046
	Median	4.1928	3.884	1.2868	3.0446
	95% HPD[3]	3.864, 4.598	3.513, 4.326	1.128,1.481	2.692, 3.383

[1]σ_s^2 = sire genetic variance; σ_e^2 = residual variance.
[2]For the zero-inflated Poisson (ZIP) and hurdle models, these refer to the count (nonbinary) component of the distributions.
[3]HPD = highest posterior density interval; values refer to lower and upper.
[4]90% probability that the true variance exceeds this value.

$$f\left(NrSC_i;\ p_{ijkl}\right) = \left(1 - p_{ijkl}\right)^{NrSC_i}\ p_{ijkl}. \qquad [5]$$

Here p_{ijkl} denotes the success probability of not generating any secondary cases (i.e., zero secondary cases) in the period assessed. The probability of success is linked to the linear predictor η_{ijkl} in Equation [1] via the logit-link function (Hadfield, 2014).

Under this model, the expected number of secondary cases associated with an index case is given by $E(NrSC_i) = (1 - p_{ijkl})/p_{ijkl}$, and the mode of this distribution is zero, indicating the most common outcome for the number of secondary cases is zero.

Model Implementation and Evaluation

All models were implemented in R using Markov chain Monte Carlo techniques within the MCMCglmm package (Hadfield, 2014). For all models, 200,000 samples were generated assuming a burn-in period of 10,000 and a thinning interval of 100. For the ZIP and hurdle Poisson models, the variances for the binary part were fixed to 1 (residual variance) and 0 (random sire effect), as residual variance for the zero-inflated process is not identifiable and the use of other priors showed incongruent and prior sensitive estimates for the random sire effect on the binary process. Otherwise, an inverse gamma prior distribution was used for the variances associated with the random genetic sire effects and the observation-level random effects associated with the count process in all models. Specifically, for the ZIP and hurdle Poisson models, the inverse Gamma scale and shape parameters were assumed equal to 0.001 for the variance of the observation-level random effects associated with the count process. For the Poisson and geometric models, the values for the shape and scale parameters were equal to 0.5 (de Villemereuil, 2012). The prior for the random sire effect used expanded parameters for prior means and variances equal to 0 and 1,000, respectively (de Villemereuil, 2012).

We evaluated the models in terms of accuracy and bias in predicting the infectivity phenotype. To this purpose, for each model, 1,000 samples were drawn from the posterior distributions of the predicted number of secondary cases associated with each index case. In rare (i.e., <0.3%) occasions, samples referred to unrealistically high values. To provide meaningful model fit statistics, predicted values greater than 500 were discarded in the model comparisons.

The root mean square error (**RMSE**) of the model predictions was then calculated as

$$RMSE_1 = \sqrt{\sum_{i=1}^{n} \frac{\left(\widehat{NrSC}_i - NrSC_i\right)^2}{n}},$$

where $\widehat{NrSC}_i$ and $NrSC_i$ represent the average value of the sampled predicted, and the observed number of secondary cases associated with index case i, respectively, and n refers to the number of index cases.

Furthermore, to take into account the uncertainty in the model predictions (i.e., dispersion of the corresponding posterior distributions), an average RMSE per index case i was also calculated as follows:

$$RMSE_2 = \frac{\sum_{i=1}^{n} \sqrt{\sum_{j=1}^{N} \frac{\left(\widehat{NrSC}_{ij} - NrSC_i\right)^2}{N}}}{n},$$

where $\widehat{NrSC}_{ij}$ represents sampled simulated value for each individual i and simulation j, N is number of simulations per individual. Prediction accuracy was further as-

Table 2. Model fit statistics for Poisson, ZIP, hurdle Poisson, and geometric models

Model	$RMSE_1$[1]	$RMSE_2$	Pearson correlation	Regression slope[2]
Poisson	4.09	7.25	0.21	0.78
ZIP	4.09	6.96	0.22	0.76
Hurdle	4.05	4.20	0.23	0.89
Geometric	4.08	6.62	0.22	0.83

[1]RMSE = root mean square error ($RMSE_1$ and $RMSE_2$ are defined in the Materials and Methods section); ZIP = zero-inflated Poisson.

[2]Regression slope closer to 1 indicates lower bias.

sessed by calculating the Pearson correlation between the average simulated $\widehat{NrSC_i}$ and observed $NrSC_i$. Likewise, bias in the model predictions was assessed using linear regression of the observed $NrSC_i$ on the corresponding average predicted values $\widehat{NrSC_i}$.

Sire estimated breeding values for infectivity were derived from sire solution of the corresponding models previously shown. In the case geometric model, probability of success of the geometric distribution refers to the probability of generating zero secondary cases, whereas the probabilities in the Poisson, ZIP, and hurdle Poisson models correspond to a given number of (nonzero) secondary cases. This implies an inverse relationship between the sire solution obtained from the geometric model and the other models. Therefore, the geometric model sire solution was multiplied by −1 to derive infectivity EBV equivalent to those from the other models. Thus, a high sire infectivity EBV for the all models refers to a high NrSC (high infectivity).

We evaluated the association between the sire infectivity EBV from each model and their daughters' phenotypes. For this purpose, we calculated the correlation of the sire infectivity EBV with the corresponding average number of observed secondary cases (i.e., average of the observed number of secondary cases associated with the index case daughters pertaining to the sire), as well as with the corresponding proportion of index case daughters that were infectious (i.e., that generated breakdowns with one or more secondary cases).

Last, we compared the models in terms of their overall concordance in the sire infectivity EBV, and in their identification of the genetically most and least infectious individuals. For the latter, we focused on the 10% most infectious and 10% least infectious sires identified by each model based on their EBV.

RESULTS

Descriptive Statistics

Figure 2a shows substantial variation in the NrSC per breakdown, although the distribution of NrSC is highly skewed with 73% of breakdowns associated with zero secondary cases. The mean number of secondary cases per breakdown was 1.024. The skewness and kurtosis for secondary cases were 18.26 and 587.09, respectively, suggesting overdispersion. The distribution of index cases per sire is illustrated in Figure 2b. Approximately 70% of the sires in the dataset were associated with just 1 index case, indicating that the majority of index cases were not closely related, as only 30% of index cases had half-sib index cases represented in this dataset.

Overdispersion and Zero Inflation

The overdispersion test of Cameron and Trivedi (1990) yielded a value of c = 9.08, indicating overdispersion in the distribution of the number of secondary cases associated with each index case. Furthermore, the ratio of the observed count of zeros in the data (4,782) over the model-predicted count of zeros (2,786) was 1.72, suggesting zero inflation. However, the posterior predictive check that compared the posterior predictive distribution of zeros from the fitted generalized linear mixed Poisson model (which also accounts for overdispersion) with the observed number of zeros suggested that a zero inflation model may not be necessary (Supplemental Figure S1).

Fixed Effects

Estimates for the fixed effects explaining significant variation in the infectivity phenotype were similar across the models. A positive association between the infectivity phenotype and the age of the index case was detected, indicating that the likelihood or severity of bTB transmission tends to increase with the age of the index case. Herd size was also positively related to the infectivity phenotype, indicating a higher likelihood and severity of bTB transmission in larger herds. Significant spatial heterogeneity in the bTB infectivity phenotype was also observed across counties. The effect of the average resistance EBV over all herd members of the index case was, however, not statistically significant in any model. However, this effect may have epidemiological significance (hence, it was included in all models), as bTB transmission might be affected by heterogeneity of herd resistance.

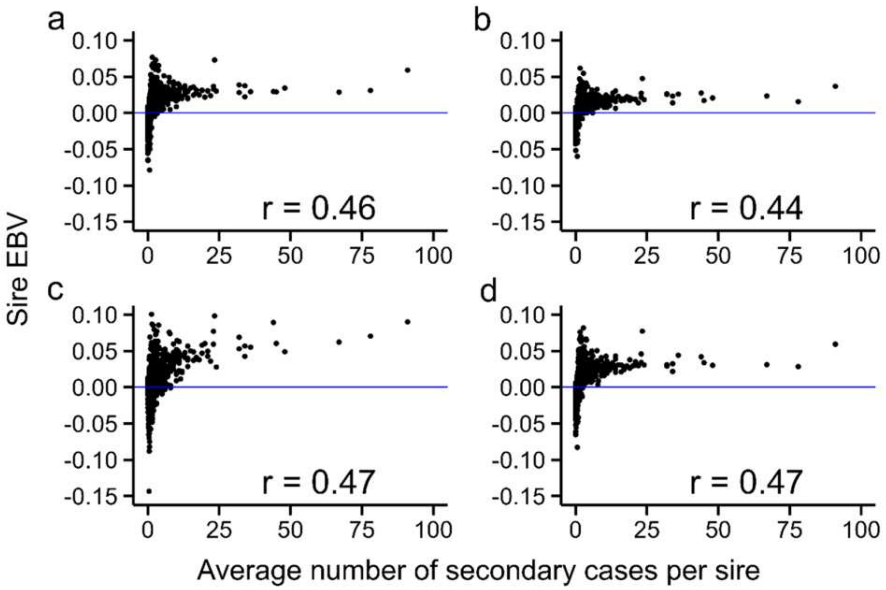

Figure 3. Infectivity EBV of index case sires plotted against their average number of secondary cases for the Poisson (a), zero-inflated Poisson (ZIP; b), hurdle (c), and geometric (d) models. The black circles represent data points for each sire, and the blue line refers to the horizontal line y = 0 to separate negative from positive sire EBVs.

Estimates of Variance Components

In Table 1, posterior distribution estimates for the variance components for the underlying linear predictors associated with the 4 different GLMMs are presented. Median sire variance estimate was largest for the hurdle model (0.0196), followed by the Poisson and geometric model (0.0164), which was almost 2 times as high as the median variance estimated using the ZIP model. Importantly, all 4 models provide evidence that there is genetic variance in cattle infectivity for bTB. Although the lower bound of the 95% highest posterior density (**HPD**) intervals (HPD 95%) for sire variance estimates were close to zero, especially for the Poisson and geometric models (variance components are constrained to be strictly positive in MCMCglmm), there was a 90% probability that the sire variance would be at least 0.0007 (Table 1 and Supplemental Figure S2, see Notes). To put this value into context, let us consider that a difference in infectivity EBV by 1 genetic SD σ_a (equal to $\sqrt{4\sigma_s^2}$, where σ_s^2 is the respective sire variance estimate) corresponds to reduction in the expected NrSC by a factor of e^{σ_a}. Then, a sire variance estimate equals to the least discernible value of 0.0007 (Table 1), implying $\sigma_a = 0.0529$, would correspond to a 5% reduction in the expected NrSC per index case.

Model Evaluation

Table 2 shows model fit statistics related to accuracy and bias of the predicted infectivity phenotypes for all 4 models. The ranking order of the models was generally consistent across the diverse statistical measures, with the hurdle Poisson model ranked best, followed by the geometric model, and the Poisson model worst (except for bias, which was similar for the Poisson and ZIP models). Specifically, similar values for $RMSE_1$ and Pearson correlation between predicted and observed NrSC were obtained for all 4 models. The correlation was relatively weak due to the large number of zero secondary cases in this dataset, making a distinction between index cases with low infectivity difficult. All 4 models had a slight tendency to overpredict the NrSC, as indicated by a slope value below 1. The biggest difference between the models was observed in the $RMSE_2$, where the hurdle Poisson model showed consistently lower deviation between observed and predicted NrSC across simulations.

All 4 models show moderately strong associations between the sire infectivity EBV and the average number of observed secondary cases attributed to the index case daughters of the sire (Figure 3). Corresponding correlations were 0.47 for the hurdle Poisson and the geometric model, and 0.46 and 0.44 for the Poisson and ZIP, respectively. It is noteworthy though that according to all 4 mod-

els, sires with negative EBV had consistently less than 10 NrSC on average (Figure 3), indicating that all 4 models can reliably identify sires with low genetic infectivity.

In contrast, sires associated with an extremely high average number of secondary cases (e.g., >25) did not necessarily have the highest infectivity EBV (Figure 3). To investigate the predictive ability of the models to identify the genetically most infectious sires, we examined the overlap between the sires with the 10% highest infectivity EBV, with the 285 (10%) sires with the highest average observed NrSC. This was 66.3%, 64.9%, 64.6%, and 62.1% for the hurdle Poisson, ZIP, geometric, and Poisson models, respectively.

The moderate association of the infectivity EBV of sires with daughter phenotypes may be caused by a large variation in the number of index cases associated with each sire, and the fact that over 70% of sires have only 1 index case. Hence, we also assessed the relationship between the sire infectivity EBV and the proportion of infectious index case daughters per sire. Moderate correlations of magnitude between 0.65 and 0.69 were observed for the Poisson, ZIP, and geometric models (Figure 4). For the hurdle Poisson model, the correlation was close to zero, as would be expected as no genetic variation was included in the binary part.

There was a strong concordance in the sire infectivity EBV between the Poisson, ZIP, and geometric models, with pairwise correlations of 0.9 or higher (Figure 5). In contrast, the magnitude of the correlations between the EBV obtained from the hurdle Poisson model and any of the other 3 models ranged between 0.36 and 0.39 (Figure 5). This substantially weaker correlation relates to the fact that the sire infectivity EBV of the hurdle Poisson model only pertain to infectious index cases with nonzero secondary cases, whereas all other models also include noninfectious index cases (i.e., those with zero NrSC) in the infectivity EBV calculations. Accordingly, agreement between the 4 models with regards to the sires with the highest genetic infectivity was relatively strong: 151 of the 285 sires with the 10% highest infectivity EBV were common between the 4 models (Supplemental Figure S3a, see Notes), and 122 (42.8%) of these also aligned with the top 10% based on observed sire average NrSC. In contrast, only 30 of the 285 sires with the lowest 10% infectivity EBV were common between the 4 models (Supplemental Figure S3b). For the Poisson, ZIP, and geometric models, the concordance in the 10% genetically most and least infectious sires was 184 (64.6%) and 133 (46.7%), respectively.

DISCUSSION

Numerous studies have implicated exploitable genetic variation in host infectivity contributing to infectious disease spread in farmed animals (Anacleto et al., 2019; Bijma et al., 2022; Dorfman et al., 2024). However, empirical genetic parameter estimates for this trait are strikingly sparse owing to a lack of both suitable data and models. Evidence for substantial genetic variation in host infectivity is, however, starting to emerge from recent experimental studies in fish (Prentice et al., 2022). Biemans et al. (2019) used associative genetic effects models to explore genetic variation in cattle infectivity for digital dermatitis. Although they obtained substantial genetic variance estimates for infectivity, models that assumed zero genetic variation in infectivity were a better statistical fit with the data, and the uncertainty in the infectivity estimates was also too large to provide conclusive evidence that genetic variance in infectivity was present and contributed significantly to the spread of digital dermatitis among cattle.

In this study, the number of secondary cases of breakdowns initiated by a single index case recorded in the national bTB database for dairy cattle genetic evaluations was used as the infectivity phenotype. Four alternative GLMM aligned with these data produced plausible estimates for sire additive genetic variance in this infectivity phenotype, providing compelling evidence that genetic variation in cattle bTB infectivity exists. The evidence was strongest for the hurdle Poisson model, which also produced the most accurate and least biased predictions in our validations. These estimates of genetic variance for infectivity on the underlying scale may appear small at first sight. However, a difference in infectivity according to EBV by 1 genetic SD σ_a corresponds to a reduction in the expected NrSC by a factor of e^{σ_a}. As such, index cases with infectivity EBV of 1 SD below the mean produce between 32% (ZIP) and 44% (hurdle Poisson) fewer NrSC than the average index case. In other words, the results of this study suggest that genetic differences in infectivity can be exploited in genetic selection toward decreasing bTB prevalence.

The association of the infectivity EBV of index case sires with the average number of secondary cases attributed to their daughters was similar across all models and moderate in size, with correlations ranging from 44% to 47%. The association between sire infectivity EBV and proportion of infectious index case daughters was slightly higher (between 65% and 69%) for the Poisson, ZIP, or geometric models. However, for the hurdle Poisson model, which only included infectious index case daughters in the genetic analyses, no such association can be made.

Of particular interest for genetic disease control is the ability to identify animals at the extreme ends of the infectivity spectrum, and in particular the model capacity to identify genetic super-spreaders, should they exist. There was a reasonable agreement between the models with

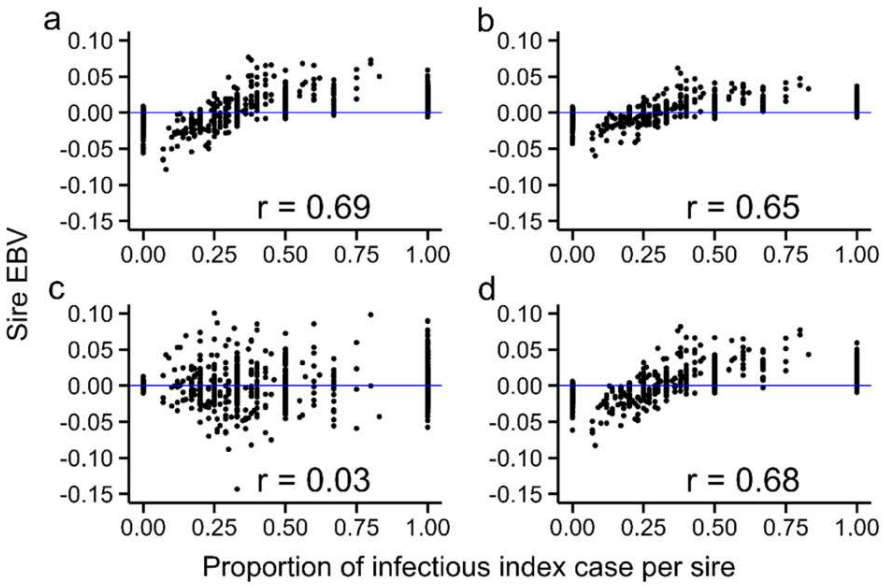

Figure 4. Infectivity EBV of index case sires against proportion of infectious daughters for the Poisson (a), zero-inflated Poisson (ZIP; b), hurdle (c), and geometric (d) models. The black circles represent data points for each sire, and the blue line refers to the horizontal line y = 0 to separate negative from positive sire EBVs.

regards to the genetically most infectious sires, with 151 (53%) out of 285 top 10% EBV sires in common, and 122 (42.8%) of these aligned also with the top 10% of sires with the highest observed average NrSC. Given that the majority of breakdowns did not result in any secondary cases within the observation window used in this study, it is difficult to identify the genetically least infectious sires from these data. Nevertheless, according to all models, sires with negative infectivity EBV had on average less than 10 associated secondary cases. This would suggest that truncated genetic selection that excludes sires with positive infectivity EBV would be a safe strategy to avoid breeding highly infectious individuals.

Several shortcomings of the infectivity phenotype used in this study need to be highlighted, as these may mask the genetic signal in infectivity. First, for infectious diseases such as bTB, it is challenging to trace back the exact source of infection. This complexity can arise from various factors, including the involvement of wildlife reservoir hosts (Broughan et al., 2016). Although previous studies have shown that cattle-to-cattle transmissions are the main source of bTB incidence in a herd (Goodchild and Clifton-Hadley, 2001; Green et al., 2008; van Tonder et al., 2021), it might not be entirely correct to assume that all observed secondary cases arise from infected index case cattle. Furthermore, over time, the probability that an animal is infected by the index case is expected to decrease, as the likelihood of new infections caused by nonindex cases increases. Although we partly mitigated this by restricting the definition of secondary cases to those identified within a ~60-d interval from the breakdown onset to the next bTB test date, it is important to acknowledge that not all animals classified as secondary cases might have been infected by the index case. However, these misclassifications would be expected to cause a downward bias in the genetic variance estimate, as they would contribute to the residual rather than the genetic variance in infectivity.

Conversely, given the potential long latency period of bTB (O'Hare et al., 2014) and the moderate sensitivity of the skin test, it is possible that all the animals infected by the index case may not have tested positive at the second test. To address this, we repeated the analyses for an extended observation period of 120 d comprising 2 consecutive tests. Although the NrSC increased slightly, the genetic variance estimates did not (results not shown), owing to a greater uncertainty regarding whether the index case was indeed the source of additional infections.

Over 70% of infectivity phenotypes in this study had a value of zero. Many factors could potentially lead to zero observed secondary cases even if the genetic infectivity of the corresponding index case was high. First, infection processes are inherently stochastic (Keeling and Rohani, 2011), implying that even if a highly infec-

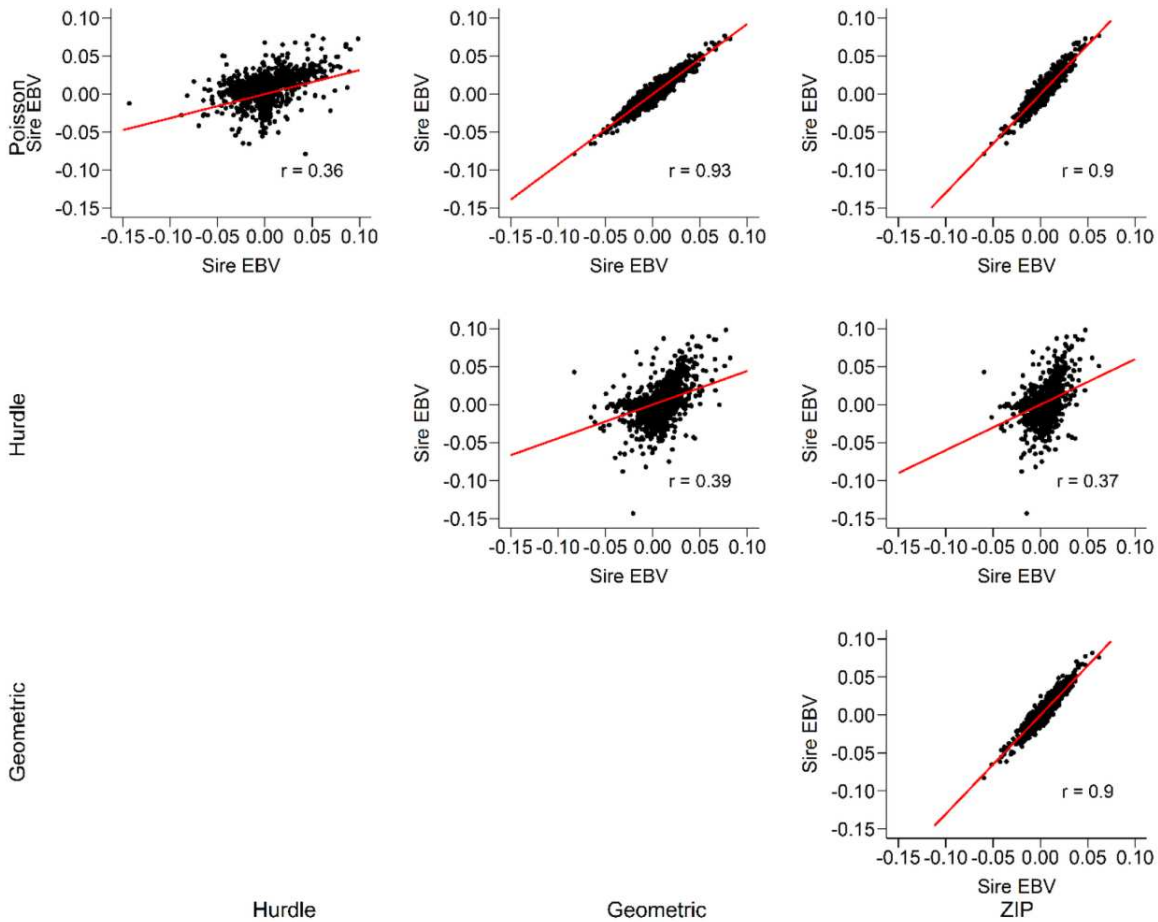

Figure 5. Pairwise correlations between EBV for sire infectivity derived from 4 statistical models: zero-inflated Poisson (ZIP), Poisson, hurdle, and geometric. Each subfigure presents the correlation coefficient between the EBV from 2 models. The red line represents the linear regression line.

tious infected individual encounters a highly susceptible noninfected individual, there is a realistic chance that transmission will not occur. Furthermore, given the long bTB latency period and the stringent bTB test and slaughter policy in the United Kingdom, it is possible that infectious index cases were removed from the herd before they had the opportunity to express their infectiousness and actually infect others.

Previous studies have identified a range of nongenetic factors contributing to observed differences in the NrSC between bTB breakdowns (Skuce et al., 2011, 2012; Byrne et al., 2017), some of which, including location, herd size, and age of the index case, were captured by the models in this study. It is possible that some variation in the NrSC due to other nongenetic factors, including differences in biosecurity levels between farms, were left unexplained by our models. However, these should not have affected the genetic variance estimate, except if they were confounded with genetic relatedness or specifically with genetic infectivity, for example, farms with higher biosecurity unknowingly keeping animals with lower genetic infectivity.

Given these shortcomings, our study provides encouraging evidence of genetic variance for bTB infectivity and demonstrates the feasibility of producing realistic EBV that may eventually underpin selective breeding for reduced disease transmission. However, further research is warranted before genetic selection for reduced bTB infectivity can be effectively implemented in practice. In particular, future research should make better use of the

existing national disease and genetic data. In this study, only data from bTB breakdowns initiated by single index cases were included in the analyses. These encompassed only 15% of all bTB breakdowns recorded nationwide between 2000 and 2022. Furthermore, the infectivity phenotype was only defined for the index cases in this study. Although index cases are the most informative individuals for detecting genetic variance in infectivity (Anacleto et al., 2019), they form a small subset (<1%) of all animals involved in bTB breakdowns. Hence, the evidence for genetic variation in infectivity in this study is based on a very small subset of potentially informative data. In our study, the precision of genetic variance estimates was compromised by the fact that only 30% of the index cases retained were paternal half-sibs. More sophisticated methods for estimating genetic parameters for infectivity using temporal disease data from all animals involved in a disease outbreak (many of which are half-sibs) are emerging (Anacleto et al., 2015; Biemans et al., 2019; Pooley et al., 2025). Although simulation studies indicate that these would provide more precise variance estimates and higher prediction accuracies than those observed here (Pooley et al., 2025), further research for adapting these to national bTB and cattle genetics data is needed. Further research should also estimate genetic correlations between bTB infectivity with bTB resistance and other economically important animal traits that are already considered in breeding programs to ensure proper integration of the new trait in the genetic selection decisions.

CONCLUSIONS

This study provides novel evidence that dairy cattle vary genetically in bTB infectivity. This implies the possibility of selectively breeding cattle with lower infectivity, which could aid in the efforts to eradicate bTB. The detection of genetic differences in infectivity among index cases is promising, especially considering that these represent only a small portion of the cattle population. Future studies that take into account the genetic variations in infectivity for all dairy cattle involved in bTB breakdowns are anticipated to reveal even more insights into the genetic factors influencing infectivity.

NOTES

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Nonstandard abbreviations used: bTB = bovine tuberculosis; GLM = generalized linear model; GLMM = generalized linear mixed models; HPD = highest posterior density; NrSC = number of secondary cases; OTF = Official bTB Free; RMSE = root mean square error; SICCT = single intradermal comparative cervical test; ZIP = zero-inflated Poisson.

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ORCIDS

D. Madenci, https://orcid.org/0009-0004-6130-9447
E. Sánchez-Molano, https://orcid.org/0000-0003-2734-8560
M. Winters, https://orcid.org/0000-0002-9491-3868
A. Mitchell, https://orcid.org/0000-0002-4059-0362
M. P. Coffey, https://orcid.org/0000-0003-4890-6218
J. D. Hadfield, https://orcid.org/0000-0002-7990-2677
G. Banos, https://orcid.org/0000-0002-7674-858X
A. Doeschl-Wilson https://orcid.org/0000-0002-2658-6973

3.3 Chapter Conclusion

This chapter demonstrated that the objective to detect genetic variability using the novel infectivity phenotype and estimate its variance components through the use of different statistical models, was successfully met and more complex models identified genetic variation signals. The finding of this chapter is an encouraging step. The next chapter builds on this by exploring how selecting for reduced bTB infectivity could impact the spread of the disease and other economically important traits.

Appendices

Appendix A : Prior Specification in Bayesian Modelling of Infectivity

The Bayesian models in Chapter 3 were implemented using MCMCglmm, as described in the published version of the chapter. This appendix provides additional detail on prior selection in the Bayesian models described in Chapter 3. Priors were selected based on standard practice in MCMCglmm and recommendations in the literature (de Villemereuil, 2012; Hadfield, 2014). For the ZIP and Hurdle Poisson models, the residual variance in the binary part was fixed to 1 and the sire variance to 0. This is because the binary process does not have an identifiable residual variance structure, and exploratory runs with alternative priors showed unstable or inconsistent results (Bolker et al., 2012; de Villemereuil, 2012). For the count process across all models, inverse gamma priors with low shape and scale values were chosen to be weakly informative as it is widely established for variance components in Bayesian models in MCMCglmm, when prior knowledge is limited (Hadfield, 2014).

Appendix B: Additional Analysis - Impact of High Number of Secondary Cases on Model Estimates

This appendix presents additional analysis comparing the original Hurdle model (best fit model) introduced in Chapter 3 with a version excluding sires with extremely high numbers of secondary cases. Specifically, sires associated with an average of more than 25 secondary cases were excluded to assess whether extreme values disproportionately influenced the estimated variance components and sire EBVs.

After removing sires with >25 average secondary cases, additional analysis was conducted to re-estimate Hurdle model using the same Bayesian framework and prior specifications described previously. Table A1 provides the posterior distribution estimates for the variance components from both versions of the model: the original Hurdle model presented in Chapter 3, and the Hurdle model excluding extreme high NSC sires. Compared to the full model, the re-estimated model showed a slightly lower sire variance. This modest reduction suggests that while extremely high secondary cases may marginally influence estimates, they do not substantially alter the overall genetic signal captured by the model.

To further explore this, Figure A1 compares the distribution of sire EBVs and their correlation with the average number of secondary cases per sire, before and after excluding sires with more than 25 average secondary cases. The results indicate that the genetic signal is not influenced by a small number of highly infectious sires. This suggests that the genetic contribution to infectivity remains consistent even when extreme cases are removed

Table A1: Posterior estimates for the variance components[1]

Variance[2]	Estimate	Model	
		Hurdle	Hurdle (>25 removed)
$\boldsymbol{\sigma_s^2}$	Mean	0.0328	0.020
	95% HPD[3]	0.0004, 0.1021	0.0002 ,0.080
$\boldsymbol{\sigma_e^2}$	Mean	1.2990	1.193
	95% HPD[3]	1.128,1.481	1.023,1.358

[1] σ_s^2=sire genetic variance, σ_e^2=residual variance
[2]These refer to the count (non-binary) component of the distributions
[3] HPD: Highest posterior density interval; values refer to lower and upper

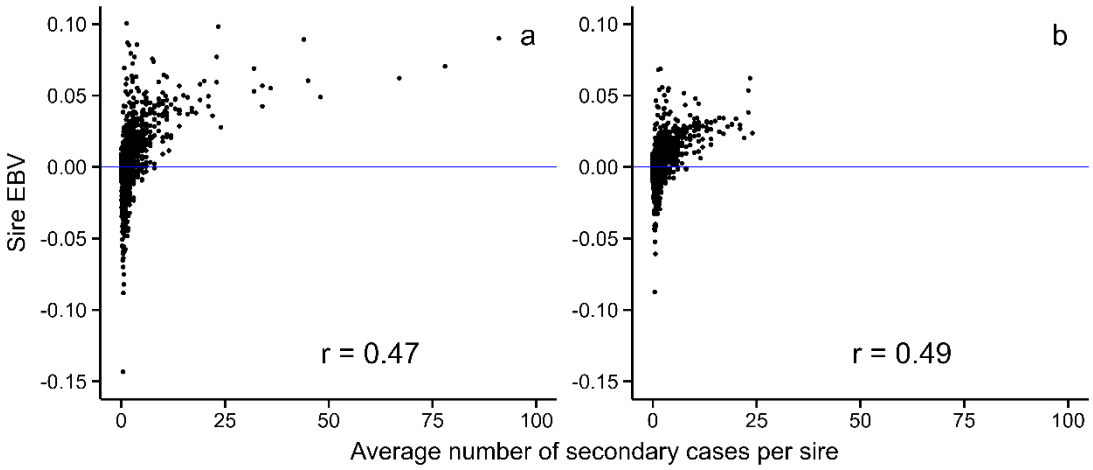

Figure A1: Infectivity EBVs of index case sires against proportion of infectious daughters for the Hurdle(a), and Hurdle (>25 removed) (b) models

Chapter 4

Implications of incorporating infectivity into breeding programs

4.1 Introduction

The previous chapter demonstrated that dairy cattle exhibit genetic variation in bovine tuberculosis (bTB) infectivity, indicating the potential for selectively breeding cattle with lower infectivity. Such a strategy could significantly contribute to efforts aimed at eradicating bTB (Banos, 2023). The present chapter demonstrates how selecting for reduced bTB infectivity may affect the disease profile as well as other economically important traits.

In dairy farming, herd performance and profitability are directly influenced by the quality of cattle genetics and management practices. Achieving profitable dairy farming requires continued improvement in productivity, health, welfare, as well as protecting genetic diversity (AHDB, 2024b). To support this vision in the UK, genetic evaluations for all major dairy breeds are produced and published three times a year, enabling the UK dairy industry to make informed decisions regarding breeding and trading. The national breeding index, called the Profitable Lifetime Index (£PLI), is used by the UK dairy industry to estimate the potential profitability of a cow over her lifetime (AHDB, 2024a). £PLI includes various production, health, and fitness traits such as milk yield, fertility index, somatic cell count (SCC), lifespan, etc., with each trait weighted

according to its relative economic importance (AHDB, 2023). Since £PLI combines numerous health and production traits, it is crucial to investigate the genetic correlations between these traits in order to achieve adequate genetic progress for the involved traits.

Many studies have investigated the genetic correlations between production, health, and fitness traits in dairy cattle, both in the UK and globally, highlighting both favourable and antagonistic relationships.

For production and health traits, Pryce et al. (1997) reported unfavourable correlations between milk yield and health conditions such as milk fever, mastitis, and lameness. Similarly, Heringstad et al. (2005) identified an antagonistic genetic relationship between susceptibility to clinical mastitis and protein yield, with a correlation of 0.43. Pérez-Cabal and Charfeddine (2013) also observed unfavourable correlations between clinical mastitis and production traits, including milk yield, fat yield, and protein yield. Further evidence was provided by Onyiro et al. (2008), who found negative correlations between digital dermatitis and traits such as lifespan, milk yield, and fat yield. Pritchard et al. (2013) supported these findings, reporting antagonistic correlations between mastitis and production traits, including milk, fat, and protein yield. In contrast, Barden et al. (2024) observed moderate favourable correlations between the Lameness Advantage Index and milk, protein, and fat yield.

For production and fertility traits, Pryce et al. (1997) found unfavourable correlations between milk yield and fertility traits. Similarly, Kadarmideen et al. (2003) and Wall et al. (2003) reported antagonistic genetic correlations between milk production and fertility traits. For fitness and fertility traits, Pryce et al. (2001); Pryce et al. (2002) highlighted antagonistic correlations between body condition score (BCS) and fertility. Wall et al. (2003) similarly identified unfavourable genetic correlations between BCS and fertility. For health and welfare traits, negative genetic correlations were observed by Onyiro et al. (2008) between digital dermatitis and lifespan. Pryce and Brotherstone (1999) reported negative correlations between lifespan and multiple health and fertility traits, including calving interval (-0.44), condition score (-0.11), mastitis occurrence (-0.22), and somatic cell count (SCC, -0.27).

Focusing on bTB, recent studies on resistance seem to suggest either advantageous or weak unfavourable correlations between bTB-related and other traits in the breeding goals related to production, welfare and fitness. Brotherstone et al. (2010) found a negative correlation between bTB susceptibility and milk yield, indicating that selecting for reduced bTB susceptibility may not have an adverse impact on milk production. Bermingham et al. (2010b) reported that genetic correlations between confirmed *M. bovis* infection and economically important traits were generally close to zero, with some unfavourable correlations with milk fat yield (0.39), SCC (-0.34), and BCS (0.36), but favourable correlations with survival (-0.62). Similarly, Berry and Bermingham (2009) identified unfavourable genetic correlations between bTB

susceptibility and traits such as SCC, fat production, and body condition score, findings that align with those of (Bermingham et al., 2010a). More recent studies have focused on broader breeding goals in relation to bTB resistance and other economically important traits. Banos et al. (2017) found that the correlations of genetic evaluations for bTB resistance with other breeding traits were mostly weak but favourable such as with £PLI, lifespan and productivity traits. In beef cattle, moderate favourable correlations were also found between bTB resistance and growth, carcass characteristics, and lifespan (Banos, 2023).

This chapter explores implications of genetic selection towards reducing bTB infectivity by:

1. Quantifying the reduction in number of secondary cases from hypothetical genetic gains achieved from selection based on sire EBVs from Chapter 3. These genetic gains are expressed as proportion of sire variance estimates from different models in Chapter 3.
2. Examining the correlations between sire EBVs for bTB infectivity and other traits including the £PLI. EBV correlations are crude proxies for genetic correlations between traits.

4.2 Materials and Methods

4.2.1. Data

The data for this chapter consist of sire EBVs for bTB infectivity, which were estimated in Chapter 3, as well as Predicted Transmitting Abilities (PTAs) for bTB resistance and a wide range of other economically important traits sourced from the Agriculture and Horticulture Development Board (AHDB, 2023).

PTAs are commonly used metrics in dairy breeding and represent the expected genetic contribution of an individual to its offspring. PTAs are calculated as half of the EBVs, as only half of an animal's genes are passed on to its progeny. This scaling does not affect the correlation calculations between different traits.

The final dataset, used to assess the correlations, included 1,904 sires present in both the bTB data used in Chapter 3 and the AHDB dataset containing 14 PTAs for production, health, welfare, and management traits.

4.2.1.1 Definition of traits

The AHDB dataset includes traits that are defined into three categories, namely, a) Production Traits, b) Health, Welfare, and Fitness Traits, and c) Management Traits (AHDB, 2023).

a) Production Traits:
    - Milk yield: Total milk produced (in kg) in 305-day lactation
    - Fat yield: The quantity of butter fat produced (in kg) in 305-day lactation
    - Protein yield: The total protein produced (in kg) in 305-day lactation

- Fat%: The percentage of butter fat in the milk
- Protein%: The percentage of protein in the milk

b) Health, Welfare, and Fitness Traits

- Fertility Index: A combination measure based on calving interval and non-return rates, providing a prediction of female fertility. Higher values indicate better fertility.
- Lifespan (days): The reduced or increased days of daughter survival. It is calculated using actual daughter survival data or proxy traits such as foot health, udder conformation, and SCC.
- Somatic Cell Count (SCC)(%): Somatic cell count is used as an indicator for susceptibility of cattle to mastitis. A lower value of -40 indicates 40% decreased SCC in a daughter.
- Mastitis (%): Calculated from farm records of actual mastitis cases. The index gives an indication of a bull's ability to transmit mastitis resistance to his daughters. This means that for every 1% decrease in a bull's Mastitis PTA, the proportion of his daughters expected to get mastitis will also decrease by 1%.
- Lameness advantage (%): Given as percentages, ranging from -5% (poor) to +5% (excellent). For every 1% increase in a bull's LA, there is a 1% decrease in the number of his daughters expected to become lame per lactation. For example, a bull with a +5% LA is expected to have 5% fewer lame daughters per lactation compared to a bull with an LA of zero.

- Digital Dermatitis (%): Ranges from about -2% to +2%, with positive values being better. A bull with a +2% DD Index is expected to have daughters with 2% fewer cases of digital dermatitis compared to a bull with a DD Index of zero.
- TB Advantage (%): ranges from -4% to +4%, with positive scores being better. For every +1 point in the index, 1% fewer daughters are expected to become infected during a TB breakdown.
- Calving Interval Index (days): Measures the interval between consecutive calving in days. For every -1 point in the index, 1 day less calving interval is expected on daughters.

c) Management Traits

- Calving ease (%): It includes both direct calving ease, which predicts the ease of birth for calves sired by a particular bull, and maternal calving ease, which reflects how easily the daughters of a sire will give birth. Expressed as “percent easy calvings” and the scale ranges from -4% to +4%, with zero being the breed average. Positive values indicate easier-than-average calvings.

4.2.2 Impact of genetic selection for reduced infectivity

To evaluate the potential impact of selecting for reduced bTB infectivity based on the sire variance estimates obtained in the previous chapter, we assessed the potential reduction in the number of secondary cases (NSC) in the

subsequent generation by converting the sire variance estimates to NSC based on the link function used in models in Chapter 3 with the following formula:

$$\Delta_{NSC}(\%) = \left|1 - e^{(\sigma_s\, i)}\right| \times 100, \quad (2)$$

where Δ_{NSC} represents the percentage change in NSC in the subsequent generation, σ_s is the sire genetic standard deviation, and i represents the proportion of the σ_s achieved per generation of selection. Then, the expected response to selection was calculated across varying proportions of σ_s.

To explore the range of possible changes in NSC under different scenarios, we computed Δ_{NSC} using posterior estimates of sire variance incorporated from the four models from the previous chapter at the 5th , 50th (median), and 95th percentiles as shown in Table 1. These percentiles were selected to examine the potential impact on NSC across a realistic range of sire variances. The 5th percentile represents a conservative, lower-end estimate of sire variance, whereas the 95th percentile represents an optimistic upper-end estimate. Together, these provide an understanding of the minimum and maximum expected impact on NSC, respectively. The 50th percentile, or median, reflects an intermediate estimate.

Table 1: Posterior estimates of sire variance

Percentiles	Models			
	Poisson	ZIP	Hurdle	Geometric
5th	0.0002	0.0009	0.0017	0.0002
50th	0.0164	0.0085	0.0196	0.0163
95th	0.1070	0.0715	0.1020	0.0976

4.2.3 Correlation between EBVs for bTB infectivity and other traits

We used the Pearson correlation coefficient to assess the relationship between EBVs for bTB infectivity and other traits as follows .

$$r_{EBV_{inf},PTA_{other}} = \frac{cov(EBV_{inf},PTA_{other})}{\sqrt{var(EBV_{inf})\,var(PTA_{other})}}, \quad (1)$$

where $cov(EBV_{inf}, PTA_{other})$ is the covariance between two trait values (i.e., EBV_{inf}, PTA_{other}) and $var(.)$ is the variance of a trait, and $r_{EBV_{inf},PTA_{other}}$ is the correlation coefficient of EBV infectivity and PTAs of other traits. A t-test was used to assess the significance of the correlations.

This assessment was conducted across four different sire groups, categorized by the number of index cases associated with each sire:

- Case 1: Consider all sires (1904 sires)
- Case 2: Consider sires with ≥2 index cases (829 sires)
- Case 3: Consider sires with ≥5 index cases (265 sires)
- Case 4: Consider sires with ≥10 index cases (110 sires)

By examining these groups, we aimed to understand how the correlation varies with the number of index cases per sire. This is crucial, as a large proportion of sires (about 60%) have only one index case, which could potentially reduce the reliability of the correlation estimates in such cases. Thus, grouping sires may help to reveal whether a stronger and more reliable correlation emerges once more informative sires are considered.

4.3 Results

4.3.1 Descriptive Statistics

Figure 1 presents the distribution of estimated breeding values for bTB infectivity trait across the four models introduced in the previous chapter: Poisson, Zero-Inflated Poisson (ZIP), Hurdle, and Geometric. The sire EBV estimates for all models fall within the range of approximately -0.15 to 0.15, where negative values represent sires with lower infectivity (i.e., favourable) and positive values indicate sires with higher infectivity. Each model slightly differs in the spread and concentration of the EBV distribution. The Hurdle model displays a distinct, sharp peak around zero, with a narrower spread compared to the other models. The Poisson and Geometric models exhibit similar distributions, with moderately broader spreads around zero. The ZIP model also peaks around zero but has a slightly less peak than the Hurdle model.

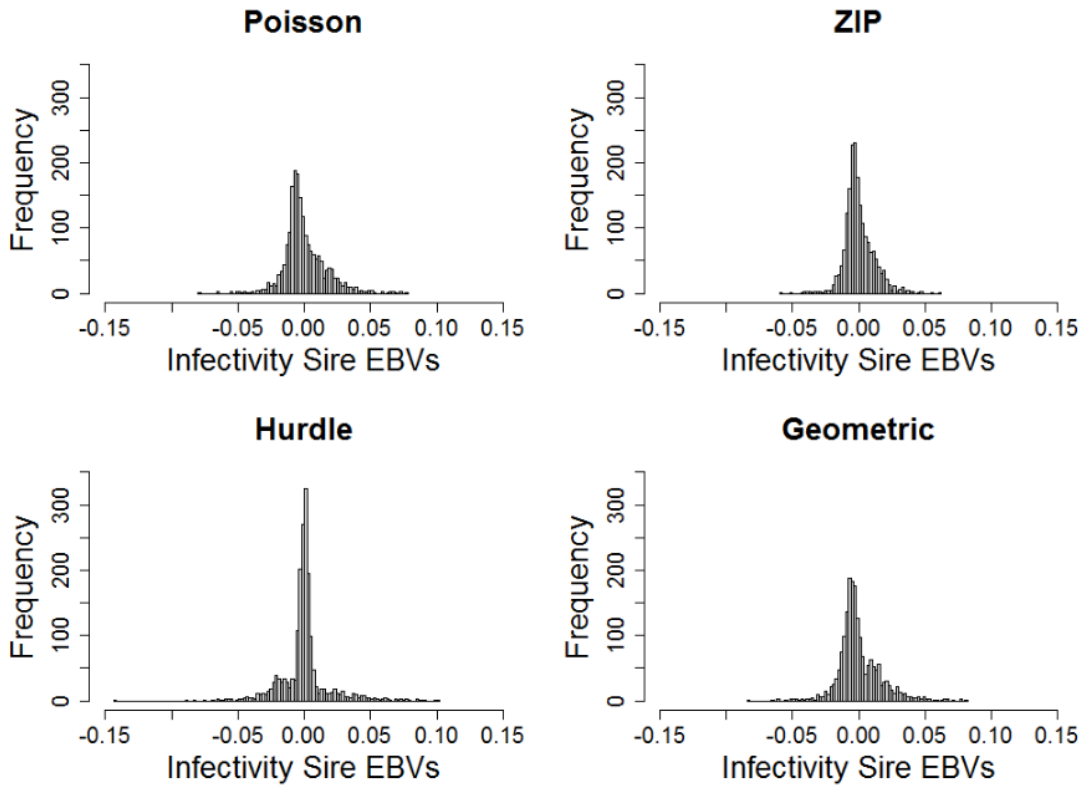

Figure 1: Histogram of infectivity EBVs for Poisson, ZIP, Hurdle and Geometric models.

Descriptive statistics of PTAs for resistance and other economically important traits for the sires of index cases are provided in Table 2. The TB Advantage trait shows a slight skew towards lower values, indicating the majority of sires has bTB resistance lower than the average. Similarly, traits associated with production, namely, milk yield, fat yield and protein yield exhibit a tendency toward negative values.

The SCC trait, on the other hand, shows a slight positive skew, indicating that higher SCC values are more prevalent in the dataset. For lifespan, most of the observed values are concentrated below zero, implying that the longevity of this population (i.e., sires of the index cases) is generally less than desirable.

Furthermore, The PLI shows a distribution skewed towards negative values, indicating that potential profitability is below average for most sires. Health-related traits, including lameness, and digital dermatitis, generally show negative mean, indicating that lower values of these health traits are more common in this population.

Table 2: Descriptive statistics of PTAs for resistance and other economically important traits for the sires of index cases

Trait	Min	1st Quar.	Median	Mean	3rd Quar.	Max
TB Advantage	-9.900	-2.025	-0.400	-0.644	1.000	9.100
Milk yield	-1485.000	-431.500	-153.000	-187.700	93.000	1308.000
Fat yield	-50.300	-16.300	-8.000	-7.052	1.800	45.300
Protein yield	-44.300	-12.300	-5.700	-5.552	1.425	40.700
Fat %	-0.370	-0.090	0.000	0.011	0.110	0.520
Protein %	-0.270	-0.040	0.010	0.010	0.060	0.270
SCC	-31.000	-3.000	5.000	5.473	14.000	54.000
Lifespan	-397.000	-116.000	-43.000	-47.100	27.000	238.000
PLI	-858.000	-320.000	-186.000	-148.750	-9.750	693.000
Fertility index	-31.900	-9.550	-3.500	-3.357	2.600	22.800
Calving interval index	-10.000	-1.000	1.000	1.694	4.000	17.000
Direct CE	-4.200	-0.500	0.200	0.146	0.900	2.800
Maternal CE	-3.100	-0.700	-0.200	-0.229	0.200	2.300
Mastitis	-5.000	-1.000	1.000	0.867	2.000	12.000
Lameness	-12.800	-2.700	-1.000	-1.074	0.700	6.800
Digital Dermatitis	-4.300	-0.900	-0.400	-0.383	0.200	2.400

4.3.2 Impact of genetic selection for reduced infectivity

To evaluate the potential impact of selecting for reduced bTB infectivity, we assessed the potential reduction in the number of secondary cases (NSC) in the subsequent generation using posterior estimates of sire variance incorporated from four models from the previous chapter at the 5th , 50th (median), and 95th percentiles as shown in Figure 2.

For the Hurdle model, a small genetic gain (i = 0.1) resulted in a median reduction of 1.41% in NSC, with values ranging from a modest 0.40% at the 5th percentile to a higher 3.25% at the 95th percentile. Under high genetic progress

(i =1), the reduction became more substantial, with a median change of 15.03%, ranging between 4.08% at the lower bound and 37.71% at the upper bound.

In contrast, the ZIP model showed smaller overall reductions. At a value of i = 0.1, the median change in NSC was 0.93%, with a range between 0.31% at the 5th percentile and 2.71% at the 95th percentile. When genetic progress increased to i =1, the reductions became 9.66% at the median, while the 5th and 95th percentiles indicated changes of 3.11% and 30.65%, respectively.

The Poisson and Geometric models exhibited similar patterns to the Hurdle model but with slight differences in magnitude. Under the Poisson model, a small genetic gain (i = 0.1) resulted in a median reduction of 1.29%, with values ranging from 0.14% at the 5th percentile to 3.33% at the 95th percentile. With a larger genetic gain (i = 1), the reductions increased to 13.66% at the median, spanning from 1.45% at the lower bound to 38.71% at the upper bound. The Geometric model yielded comparable results, with a median reduction of 1.28% for i = 0.1, increasing to 13.62% under i =1. The 5th and 95th percentile estimates for the Geometric model ranged between 0.36% and 3.17% for small genetic progress and between 3.17% and 36.68% for high genetic progress.

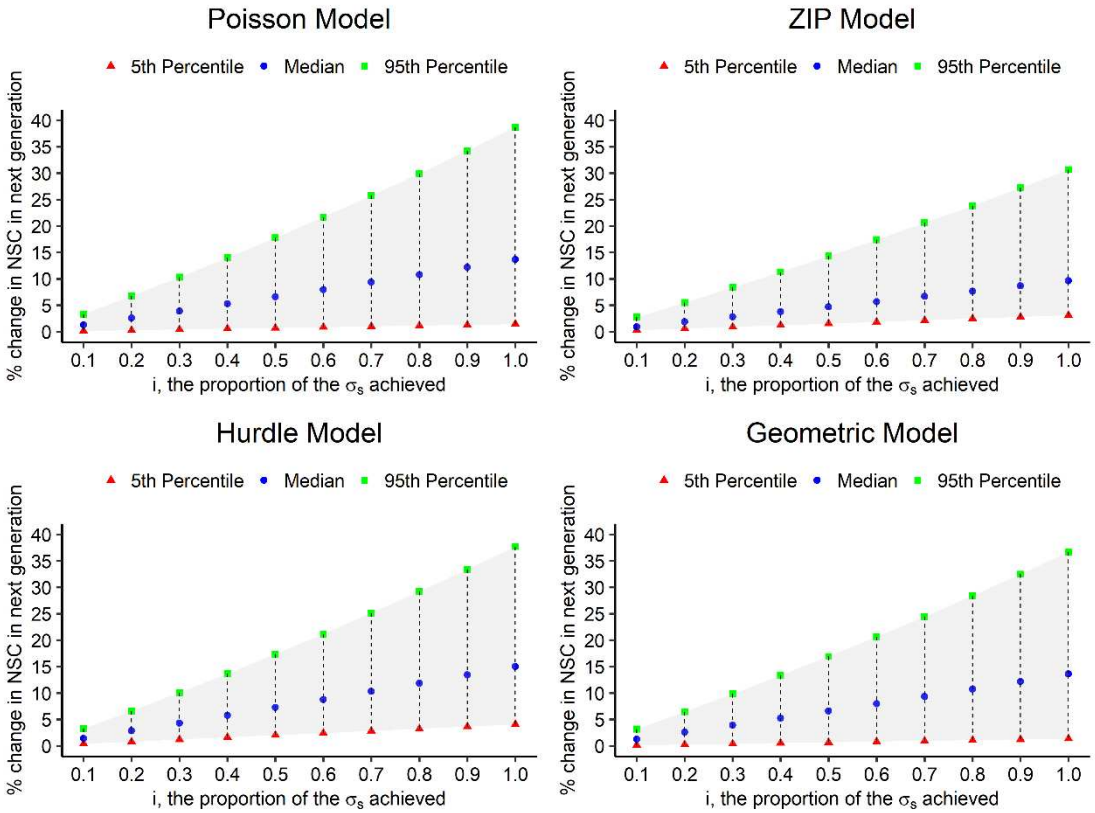

Figure 2: Impact of selection for reduced bTB infectivity on the expected percentage reduction in the NSC per index case, in the next generation. Symbols represent percentiles in sire variance estimates, and the areas shaded in grey represent the 50% credibility estimate for the expected change in NSC.

4.3.3 Correlation between EBV infectivity and other traits

We used estimated breeding values for bTB infectivity from Chapter 3 and evaluated the correlations between EBVs for bTB infectivity and other production and health traits, using different models (Hurdle, ZIP, Geometric, and Poisson). The correlations were analysed across different thresholds for the number of index cases per sire (all sires, sires with ≥2, ≥5, and ≥10 index cases) as shown in Table 3-6. Overall, the correlations between infectivity and all other health traits, as well as production and fertility traits were generally low across the models and thresholds where the magnitude of correlations were

generally below 0.1. For most traits, the correlations were not statistically significantly different from zero, with a few exceptions. Protein yield showed modest but unfavourable correlations with infectivity at higher thresholds (≥5 or ≥10 index cases), especially under the ZIP (0.139 and 0.229), Poisson (0.138 and 0.208) and Geometric (0.143 and 0.228) models. In addition to protein yield, milk yield also showed unfavourable modest correlations (ranging from 0.119 to 0.131) in ZIP, Poisson, and Geometric models at Case 3 (sires with ≥5 index cases). Similarly, the calving interval index showed significant unfavourable correlations at higher thresholds under these models. In addition, lameness showed modest and unfavourable correlation (0.182) in Hurdle model at Case 4 (sires with ≥10 index cases).

Across all four models (Hurdle, ZIP, Poisson, and Geometric), a consistent trend was observed where the magnitude of correlations increases as the higher reliability sires considered, moving from Case 1 (all sires) to Case 4 (sires with ≥10 index cases). Traits such as protein yield, milk yield, and lameness show notably stronger correlations in Case 4, with most of them reaching statistical significance.

Table 3: Correlations between bTB infectivity EBVs and other traits - Hurdle model

Traits	Case 1†	Case 2†	Case 3†	Case 4†
TB Advantage	0.008	0.022	0.044	0.055
Milk yield	-0.012	-0.028	0.003	-0.023
Fat yield	0.012	-0.001	0.051	0.085
Protein yield	0.002	-0.012	0.055	0.091
Fat % lactation	0.034	0.039	0.052	0.117
Protein % lactation	0.032	0.038	0.080	0.169
SCC	-0.010	-0.010	-0.005	-0.102
Lifespan	0.029	0.045	0.050	0.089
PLI	0.023	0.024	0.044	0.120
Fertility index	0.006	0.014	-0.013	0.027
Calving interval index	-0.004	-0.021	0.005	-0.074
Direct CE	-0.003	0.001	0.027	0.096
Maternal CE	-0.010	0.008	0.042	0.122
Mastitis	-0.029	-0.042	-0.013	-0.124
Lameness	0.037	0.046	0.078	0.182*
Digital Dermatitis	0.011	0.011	0.050	0.137

*$P < 0.05$. Negative correlations are favourable except for SCC, calving interval and mastitis

†Case 1: all sires, Case 2: sires with ≥2 index cases, Case 3: sires with ≥5 index cases, Case 4: sires with ≥10 index cases

Table 4: Correlations between bTB infectivity EBVs and other traits - ZIP model

Traits	Case 1†	Case 2†	Case 3†	Case 4†
TB Advantage	0.016	0.047	0.092	0.161
Milk yield	0.007	0.027	0.119*	0.163
Fat yield	0.008	0.032	0.106	0.144
Protein yield	0.014	0.042	0.139*	0.229*
Fat % lactation	-0.001	-0.001	-0.041	-0.052
Protein % lactation	0.010	0.016	-0.010	0.031
SCC	-0.014	-0.021	-0.039	-0.174
Lifespan	-0.027	-0.038	-0.068	0.047
PLI	-0.011	-0.003	0.014	0.165
Fertility index	-0.019	-0.003	-0.016	0.136
Calving interval index	0.020	0.001	-0.003	-0.203*
Direct CE	0.001	-0.017	-0.009	0.128
Maternal CE	-0.004	0.004	-0.074	-0.053
Mastitis	-0.007	-0.001	0.037	-0.100
Lameness	-0.011	-0.043	-0.005	0.030
Digital Dermatitis	-0.007	-0.015	0.046	0.085

*$P < 0.05$. Negative correlations are favourable except for SCC, calving interval and mastitis

†Case 1: all sires, Case 2: sires with ≥2 index cases, Case 3: sires with ≥5 index cases, Case 4: sires with ≥10 index cases

Table 5: Correlations between bTB infectivity EBVs and other traits - Poisson model

Traits	Case 1†	Case 2†	Case 3†	Case 4†
TB Advantage	0.012	0.056	0.067	0.152
Milk yield	0.024	0.054	0.131*	0.163
Fat yield	0.023	0.042	0.082	0.088
Protein yield	0.028	0.061	0.138*	0.208*
Fat % lactation	-0.005	-0.028	-0.083	-0.108
Protein % lactation	0.002	-0.006	-0.036	0.0001
SCC	-0.006	-0.011	-0.017	-0.175
Lifespan	-0.028	-0.046	-0.073	0.041
PLI	-0.003	-0.003	-0.002	0.140
Fertility index	-0.014	-0.003	-0.001	0.163
Calving interval index	0.012	0.000	-0.014	-0.231*
Direct CE	0.018	0.003	0.000	0.127
Maternal CE	0.000	0.013	-0.055	-0.022
Mastitis	0.011	0.022	0.065	-0.067
Lameness	0.002	-0.032	-0.005	0.035
Digital Dermatitis	0.009	-0.004	0.051	0.090

*$P < 0.05$. Negative correlations are favourable except for SCC, calving interval and mastitis

†Case 1: all sires, Case 2: sires with ≥2 index cases, Case 3: sires with ≥5 index cases, Case 4: sires with ≥10 index cases

Table 6: Correlations between bTB infectivity EBVs and other traits – Geometric model

Traits	Case 1†	Case 2†	Case 3†	Case 4†
TB Advantage	0.019	0.054	0.081	0.151
Milk yield	0.016	0.049	0.129*	0.177
Fat yield	0.019	0.043	0.091	0.115
Protein yield	0.025	0.063	0.143*	0.228*
Fat % lactation	0.000	-0.020	-0.070	-0.099
Protein % lactation	0.012	0.006	-0.023	0.004
SCC	-0.013	-0.019	-0.017	-0.152
Lifespan	-0.018	-0.023	-0.074	0.036
PLI	0.005	0.017	0.005	0.149
Fertility index	-0.007	0.007	-0.005	0.162
Calving interval index	0.007	-0.014	-0.017	-0.236*
Direct CE	0.015	0.007	-0.005	0.119
Maternal CE	0.008	0.026	-0.059	-0.012
Mastitis	-0.001	0.007	0.068	-0.055
Lameness	0.015	-0.015	-0.003	0.043
Digital Dermatitis	0.012	0.008	0.064	0.106

*$P < 0.05$. Negative correlations are favourable except for SCC, calving interval and mastitis

†Case 1: all sires, Case 2: sires with ≥2 index cases, Case 3: sires with ≥5 index cases, Case 4: sires with ≥10 index cases

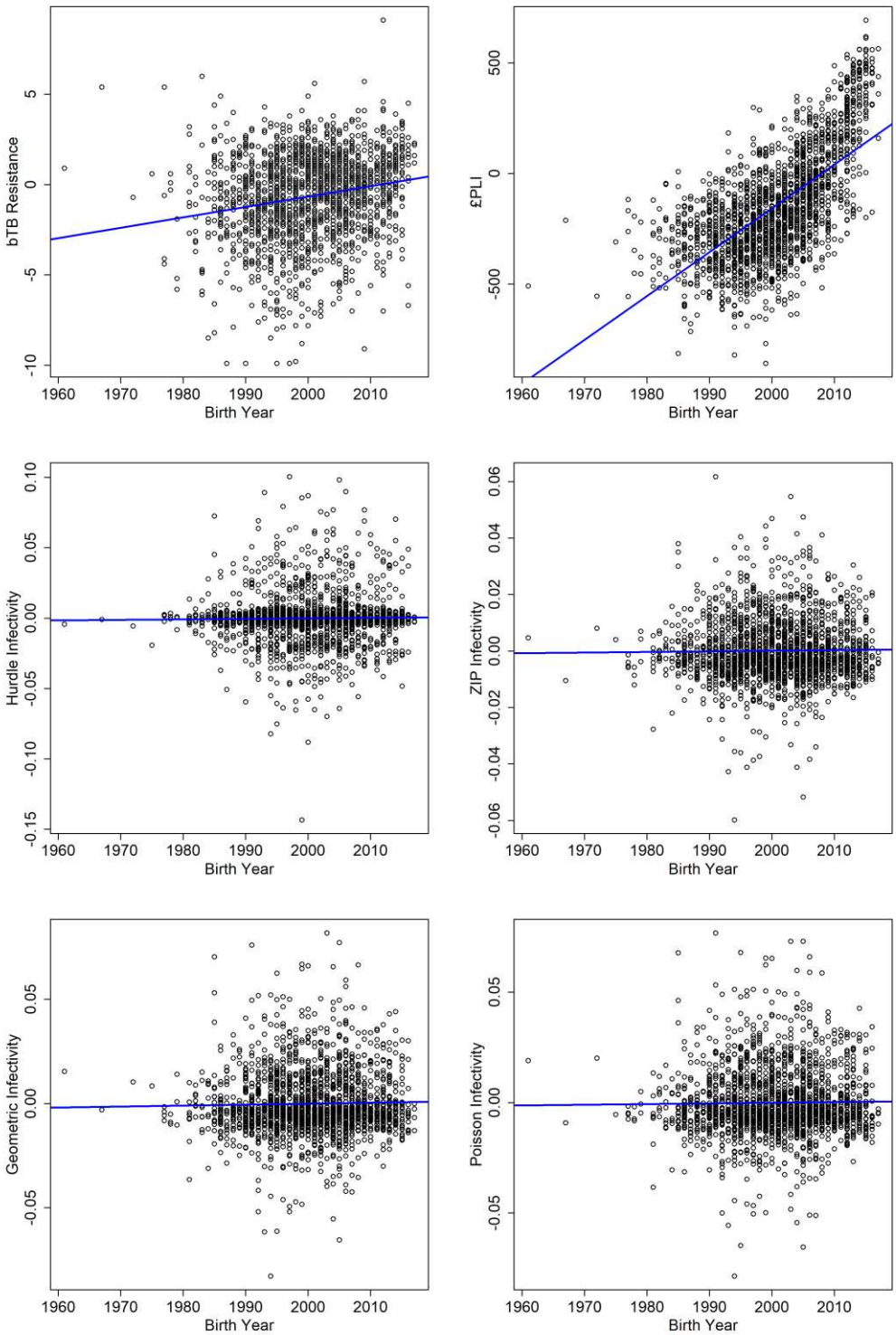

Figure 3: The change of economically important traits (EBVs of TB Advantage and £PLI) over years compared with the infectivity EBVs across four models. The birth year corresponds to the birth year of sires used in the present study.

Figure 3 shows trends in EBVs of TB Advantage and £PLI, alongside bTB infectivity EBVs across four models: Hurdle, ZIP, Geometric, and Poisson, over time. The results clearly illustrated that, due to selective breeding efforts, £PLI and TB Advantage have shown consistent improvement over years, reflecting the genetic progress achieved for these traits.

In contrast, no trend was observed in bTB infectivity EBVs across all four models, suggesting that selection for £PLI and TB Advantage has not influenced bTB infectivity. This observation aligns with our earlier findings, which indicated no significant genetic correlations between bTB infectivity and other economically important traits. These results highlight that reducing bTB infectivity would require direct selection, as it does not appear to be affected by current selection practices for production and health traits.

4.4 Discussion

In this chapter, we explored the implications of incorporating bTB infectivity into breeding programs, focusing specifically on the potential reduction in NSC when selecting for reduced infectivity. Furthermore, the correlations between sire EBVs for bTB infectivity and other traits were explored. These include both bTB resistance and economically important traits such as milk yield, protein yield, and calving interval.

Our results indicated that selecting for reduced bTB infectivity has the potential to significantly reduce NSC over generations, but the magnitude of this

reduction depends heavily on the genetic variance or heritability of a trait, as well as prediction accuracy and selection intensity. Typically, the response to selection is calculated using the breeder's equation. In this study, we did not explicitly use the breeder's equation because we lack estimates for prediction accuracy, which reflect how well an animal's EBV predicts its true genetic merit. Prediction accuracy is crucial for reliable estimates of genetic response, and without it, applying the breeder's equation would be incomplete and potentially misleading. Nonetheless, the trends observed in our results still provide valuable insights into the potential reductions in NSC achievable through targeted selection for reduced bTB infectivity.

Our results provided a range of possible reductions in NSC, from modest to substantial. Achieving a 10% reduction in the expected NSC, for instance, would require strong genetic progress ($i > 0.8$). This typically requires at least moderate selection intensity, or high genetic variance for the trait in consideration. However, in our case, the relatively low genetic variance estimates for infectivity obtained in our study implies that reaching substantial reductions would require strong selection intensity. If selection intensity is not sufficiently strong, the reduction in infectivity will be likely slower than expected from previous simulation studies (Tsairidou et al., 2018).

An additional aspect covered in this chapter is the correlations between sire EBVs for bTB infectivity and other traits. The results revealed that most correlations between bTB infectivity EBVs and economically important traits were not statistically significant and were generally close to zero. This suggests

that bTB infectivity may be genetically independent to other traits, and thus not strongly compromise genetic improvement in other important traits. On the other hand, given the weak correlations, improvements in infectivity are unlikely to occur as an unintended consequence of selection for other traits. In other words, if the goal is to reduce bTB infectivity, it would require explicit selection for this trait. However, when examining data from more informative sires (Case 3 and Case 4), we observed weak, yet unfavourable correlations between bTB infectivity and certain production traits, such as milk and protein yield, as well as calving interval. These weak correlations were consistent across all models except for the Hurdle model, which showed a weak unfavourable correlation between bTB infectivity and lameness.

While these results align with previous studies showing unfavourable correlations between health and production traits traits (Onyiro et al., 2008; Pritchard et al., 2013), they differ from studies that report favourable genetic correlations between bTB resistance and production traits (Brotherstone et al., 2010; Banos et al., 2017). In our case, we found no evidence to suggest that similar favourable correlations apply to the bTB infectivity and production traits.

However, the reliability of the EBV correlation estimates might be compromised due to several limitations of the present study. For example, many sires in the data had only one daughter, which might lead to inaccurate EBVs, and in turn, inaccurate correlations with other traits. This limited accuracy makes it difficult to definitively conclude that no, or unfavourable correlations exist between bTB infectivity and production traits.

Furthermore, another shortcoming would be the characteristics of the sires included in the analysis. Descriptive statistics of traits for sires of index cases (see Table 2) suggested that these sires included in our dataset (i.e. those with bTB index cases) may not be random samples from the population, as the mean EBVs for bTB resistance and other traits were not zero. Since the available trait data from AHDB is standardized with an expected mean of zero, we would anticipate an even distribution around this mean in an unbiased sample. However, in our subset, values are skewed toward less favourable sires, which implies that the sires included in this analysis may be genetically less resistant to bTB and other diseases, and generally less productive and fit. This skew towards unfavourable sires in our sample may affect the reliability of the correlation estimates.

Although our results do not provide strong evidence that selecting for reduced bTB infectivity would have favourable impacts on other economically important traits, they equally do not suggest any antagonistic effects, considering limitations and shortcomings. In other words, selecting for bTB infectivity does not appear to negatively impact other traits. Therefore, our approach serves as an important preliminary step toward understanding the genetic relationships between bTB infectivity and other traits.

While the ultimate goal is to assess genetic correlations, deriving accurate unbiased estimates for these would require the use of multivariate models. Extending the generalized linear mixed models for estimating genetic parameters for bTB infectivity in Chapter 3 to multivariate models is however

beyond the scope of this thesis. However, the investigated EBV correlations aim to offer an initial insight on the relationships between bTB infectivity and other traits.

4.5 Conclusion

This chapter provides insights into future selection for bTB infectivity using the trait definition and EBVs derived in the present thesis. This is a first attempt to assess impact of selecting for bTB infectivity on the trait profile and other economically important traits in subsequent generations. It is not possible to know how much emphasis future breeding programmes will place on bTB infectivity. Our results suggest that even with little emphasis, a small to modest benefit may be expected. Results also indicate that selecting for reduced bTB infectivity would not compromise other animal traits in the current breeding goal. The reverse is also true, as current genetic selection practices do not seem to affect bTB infectivity.

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Chapter 5

General Discussion

5.1 Summary of the Findings

The research presented in this thesis makes a novel contribution by estimating genetic variation in bTB infectivity using field data for the first time. The overriding contribution includes the definition of a novel bTB infectivity phenotype and evidence of genetic variation.

Specifically, Chapter 2 introduced the "index case approach" to define bTB infectivity. Using the number of secondary cases (NSC) attributed to the index case as a proxy phenotype for infectivity, the chapter employed linear and generalized linear mixed models (GLMMs) to assess genetic variation. Furthermore, we investigated potential non-genetic effects that contribute to explaining variability in bTB infectivity. Results provided the first evidence that bTB infectivity exhibits genetic variation, however, these findings also highlighted the need for more advanced statistical models to accurately capture this variation.

Chapter 3 addressed the limitations in Chapter 2 by applying MCMC techniques to fit advanced GLMMs that handle potential overdispersion and zero inflation effectively. Multiple models were tested, including Poisson, Zero-Inflated Poisson, Hurdle Poisson, and Geometric models. The results validated the genetic basis of bTB infectivity and showed preliminary findings that selecting

for reduced infectivity can contribute to lowering the number of secondary cases. This chapter also derived estimated breeding values (EBVs) for sires of the index case.

Using the EBVs from Chapter 3, Chapter 4 evaluated the practical implications of selecting for reduced bTB infectivity in subsequent cattle generations. Results suggested that a notable reduction in secondary cases can be achieved, demonstrating the potential impact of genetic selection for reduced bTB infectivity. Furthermore, Chapter 4 examined the EBV correlations between bTB infectivity and other economically important traits to evaluate the impact of selecting for reduced bTB infectivity on traits currently in use in breeding programs. The results revealed no antagonistic relationships between sire EBVs for bTB infectivity and other economically important traits.

5.2 Implications

This thesis demonstrates, for the first time using field data, that genetic variation exists in bTB infectivity, and genetic selection for reduced infectivity can lead to a decrease in the number of secondary cases (NSC) in subsequent generations. The selection of sires with low infectivity breeding values could make it possible to reduce the overall transmission potential within herds over successive generations. Genetic-epidemiological simulation studies have demonstrated that such an approach could complement current genetic selection and accelerate bTB eradication (Tsairidou et al., 2018; Banos, 2023).

Furthermore, this thesis provides the first evidence that selection for reduced bTB infectivity does not negatively impact other economically important traits. The absence of antagonistic correlations between sire EBVs for infectivity and other economically important traits is a promising finding. This alleviates concerns about potential trade-offs that could undermine the broader adoption of genetic selection for disease traits. Farmers and breeding organizations can integrate bTB infectivity into selection criteria without sacrificing productivity, aligning with industry priorities for economically sustainable solutions.

5.3 Limitations and Challenges

5.3.1 Index Case Approach

In infectious diseases, infectivity refers to the ability of an individual to transmit the disease once infected (Lipschutz-Powell et al., 2012; Knap & Doeschl-Wilson, 2020). In the case of bTB, defining the infectivity phenotype is particularly challenging due to the disease's complex epidemiology (Anacleto et al., 2015; Tsairidou et al., 2018). Transmission occurs through multiple routes, including direct contact between animals and indirect contact via contaminated environments (Broughan et al., 2016). Additionally, wildlife reservoirs contribute to the spread of the disease, complicating the identification of infection sources (Allen et al., 2018). The prolonged incubation period of bTB further complicates the definition of infectivity phenotypes since infected animals may not exhibit clinical signs for extended periods, during which they

can still transmit the pathogen to others. This latency makes it difficult to determine when an animal became infectious and to identify the exact transmission pathways (O'Hare et al., 2014).

In this thesis, we introduced the index case approach to define a novel infectivity phenotype. This phenotype can be derived based on existing data and no new data need to be collected. The developed infectivity phenotype enabled us to capture significant genetic variation and show that the bTB infectivity is partly controlled by host genetics. Although this approach is sufficient to explain genetic variation for bTB infectivity, it also has some limitations. In the analyses, only bTB breakdowns initiated by single index cases were included to accurately attribute the NSC to the index case. This subset represents 15% of all breakdowns and <1% of all animals involved in bTB breakdowns recorded nationally between 2000 and 2022. While index cases are highly informative for detecting genetic variance in infectivity (Anacleto et al., 2019), relying on such a small subset may limit the broader applicability of the findings.

5.3.2 Models

The models employed in this thesis produced plausible estimates of sire genetic variance for the bTB infectivity phenotype, providing compelling evidence that genetic variation in cattle bTB infectivity exists. This successfully addresses the key question of whether genetic variation in bTB infectivity exists. Additionally, the models estimated sire EBVs, offering critical insights into the practical

implications of selecting for reduced bTB infectivity and the relationships between this trait and other economically important traits.

Despite these achievements, there are notable limitations to the current models. While the estimates of genetic variance for infectivity on the underlying scale are valid, the models do not currently support the calculation of heritability estimates or the reliability of EBVs. This limitation restricts the direct application of the breeder's equation for predicting response to selection and precise calculations of genetic correlations with other traits. As a result, while the findings are significant, they do not yet provide the complete framework needed for immediate integration into breeding programs.

Nonetheless, this thesis represents a crucial initial step toward integrating bTB infectivity into breeding objectives, laying the groundwork for future advancements in this area.

5.4 Future Work

5.4.1 Refining Case Definitions

This thesis relies on case definition derived from a combination of skin test results, post-mortem visible lesions, and *M. bovis* culture findings (Banos et al., 2017). While these are useful, they may not capture all bTB cases considering their limitations.

Future research could explore refining the bTB case definition by integrating more advanced diagnostics with improved sensitivity. For instance, the IFN-γ test can be integrated for a more robust case definition to mitigate false negative rates and, in turn, enable the detection of infected animals more accurately. Additionally, novel approaches using machine learning techniques, such as Deep Learning, show promise in deriving cost-effective and accurate bTB status prediction. Recent research has demonstrated that combining extensive mid-infrared (MIR) spectral data from routine milk recording with bTB surveillance records can enable the prediction of individual animals' bTB status, with high specificity, sensitivity, and accuracy of 0.94, 0.96, and 0.95, respectively (Denholm et al., 2020). These methods may also complement improving case definitions to accurately detect infected animals.

In addition to refining case definitions mentioned above, our data also provide an opportunity to explore the relationship between post-mortem lesion status of index cases and the number of secondary cases they generated. Among the index cases included in this study, approximately 34% were confirmed to have

visible lesions at slaughter. These animals generated, on average, a higher number of secondary cases compared to those without visible lesions. This observation supports the hypothesis that animals exhibiting gross pathology may be more infectious and raises the possibility that skin-test positive cattle with and without lesions may represent distinct host phenotypes. Some recent findings suggest these groups may reflect different, genetically determined host responses (Wilkinson et al., 2017). This warrants further investigation into whether the genetic predisposition to develop lesions is associated with increased transmission risk, and whether combining lesion scores with infectivity estimates could provide deeper insight into the pathogen transmission and support more refined selection strategies.

5.4.2 Examining Additional Risk Factors

Previous studies have highlighted various non-genetic factors that influence differences in the number of secondary cases (NSC) across bTB breakdowns (Skuce et al., 2011; Skuce et al., 2012; Byrne et al., 2017; Allen et al., 2018). In this thesis, factors such as location, herd size, and the age of the index case were accounted for in the models. However, it remains possible that other non-genetic variables, not included in the analysis, may also contribute to the observed variation in NSC. This underscores the importance of considering additional non-genetic effects in future modelling efforts.

One important consideration would be the role of animal density within herds. While herd size at the start of a breakdown is already included in our analyses,

it might not fully capture the effect of contact rates, which are influenced by the spatial distribution of animals within a herd. Higher animal density can facilitate the transmission of infectious agents. It may be calculated as Density=Herd Size/Land Area (in hectares or acres) if the land area information is available (Smith et al., 2006; Djelouadji et al., 2011; Skuce et al., 2011; Allen et al., 2018).

Another critical factor is the interaction with wildlife. In Great Britain, badgers are a well-known reservoir of bTB. Farms with higher levels of wildlife interaction may face an increased risk of primary infections, which could, in turn, affect the NSC. While wildlife interaction is unlikely to directly influence genetic infectivity, it could cause variability in transmission dynamics that needs to be accounted for (Courtenay et al., 2006; Broughan et al., 2016).

Environmental variables such as climate and geography are also worth considering. Factors like temperature, humidity, and soil type can influence the survival and persistence of *M. bovis* in the environment (Maddock, 1933; Courtenay et al., 2006; Barbier et al., 2017; Kaneene et al., 2017; Allen et al., 2018), subsequently affecting infection patterns (Byrne et al., 2015). For instance, *M. bovis* has been shown to persist longer in cool, moist, and shaded conditions (Rodríguez-Hernández et al., 2016). Additionally, VanderWeele et al. (2013) have documented the potential for environmental confounding in gene-environment interaction, highlighting the importance of accounting for environmental variables to avoid misleading conclusions. Therefore, incorporating these environmental factors into models could help reduce unexplained variability and provide a more comprehensive understanding.

Furthermore, the variation in the pathogen may also play a significant role in host infectivity. Evidence from human TB research suggests that different Mycobacterium tuberculosis lineages vary in their ability to cause disease and spread. For example, the Beijing lineage has been shown to be more virulent and transmissible compared to others (Coscolla & Gagneux, 2014; Holt et al., 2018). Since pathology is thought to be a key driver of onward transmission in TB, it is plausible that more virulent *M. bovis* strains may also increase the likelihood of an infected animal becoming highly infectious. This may be supported by our preliminary findings that index cases with visible lesions were associated with higher NSC on average, and by previous work showing variation in *M. bovis* virulence (Wright et al., 2013). Allen (2017) has also raised the possibility on the role of bacterial lineage in host infectivity, suggesting that host–pathogen interactions may contribute more to variation than host genetics alone (Woolhouse et al., 2002).

Further support for this idea comes from genomic studies of *M. tuberculosis*. Comas et al. (2010) showed that genes encoding T cell antigens, which are the key components of the host adaptive immune response, are among the most conserved regions of the *M. tuberculosis* genome. This suggests that TB bacilli may have evolved to interact with the host's adaptive immune system to promote pathology and increase transmission. In that sense, a highly infectious animal may have adaptive immune variation, which in turn could drive the development of pathology and increase secondary transmission. Therefore, incorporating pathogen genomic data into future models may also help to improve the definition of the infectivity phenotype. For instance, true secondary

cases are expected to share the same *M. bovis* sub-lineage as the index case. If different sub-lineages are present within a herd, some apparent secondary cases may not actually reflect true transmission events. Filtering cases based on pathogen lineage could therefore help refining phenotype definitions. However, using genomic data to infer precise transmission chains remains challenging. Due to the slow mutation rate of *M. bovis*, outbreaks are often highly genetically similar for pathogen, limiting the ability to resolve who infected whom (Akhmetova et al., 2023). However, approaches that combine pathogen genome data with epidemiological metadata have shown promise in inferring more likely transmission routes (Rossi et al., 2022; Wood et al., 2024).

5.4.3 Multivariate Approaches for Genetic Correlations

This thesis examined the correlations between sire EBVs for bTB infectivity and other economically important traits. While these EBV correlations provide an initial understanding of the relationships between traits, they serve as proxies rather than direct measures of genetic correlations. Accurately estimating genetic correlations would require the application of multivariate models, which is beyond the scope of this thesis. Future research should focus on extending the models used in this thesis to multivariate approaches. For instance, Bayesian approaches such as those implemented in MCMCglmm (Hadfield, 2010) allow for the simultaneous analysis of multiple traits and models could provide unbiased estimates of genetic correlations. Such efforts would offer a

more precise understanding of the genetic relationships between bTB infectivity and other traits.

Although these proxies (the correlations between sire EBVs for bTB infectivity and bTB resistance and other economically important traits) are not direct measures of genetic correlations, one particularly interesting outcome of this work was the apparent lack of correlation between infectivity and resistance phenotypes. This finding raises the possibility that these two traits may be governed by different underlying biological or genetic mechanisms. The resistance phenotype used in TB Advantage is thought to reflect the strength of the innate immune response to TB (Bermingham et al., 2014; Banos et al., 2017). Given this, it is worth considering whether infectivity may be influenced more heavily by the adaptive immune response instead of innate immune response and the variation in adaptive immune response may partially be under genetic control. If true, this would suggest that infectivity and resistance are influenced by separate aspects of host immunity, potentially under different genetic control. While further research is needed to explore this possibility, it provides a valuable direction for future research and may help explain the observed lack of association between these traits.

5.4.4 Expanding Across Breeds

The methodologies and findings presented in this thesis, which focus on genetic variation in bTB infectivity within Holstein Friesian cattle, have broader applicability to other dairy breeds and beef cattle populations. Expanding this

research to other dairy breeds or beef cattle could provide valuable insights into genetic variation in infectivity. Investigating both dairy and beef breeds would, therefore, enable a comprehensive understanding of genetic factors underlying bTB transmission across the cattle industry. Such studies would offer a more complete picture of genetic factors affecting bTB transmission across all cattle breeds, enhancing the applicability and impact of the methodologies developed in this thesis (Banos, 2023).

5.4.5 Advanced Genetic Models

To address the limitation of the index case approach, future work could explore advanced genetic models that allow for a more comprehensive estimation of genetic parameters associated with infectivity. A promising direction involves applying tools like the recently developed SIRE (Susceptibility, Infectivity, Recoverability Estimator) software (Pooley et al., 2024), which is currently designed to estimate polygenic contributions to host traits using temporal epidemic data. Unlike the index case approach, this framework can analyse genetic and non-genetic effects for all individuals within a population, not just those identified as index cases. The original version of the software implements a stochastic genetic-epidemiological SIR (Susceptible-Infectious-Recovered) model, which incorporates genetic (co-)variance in susceptibility and infectivity, as well as in recoverability. Similar to the models for the infectivity phenotype assumed in this thesis, SIRE models the infection process of individuals as a Poisson process. The software adopts a Bayesian inference framework, which

implies that it can handle uncertainties in the data, such as irregular disease diagnostics or unknown infection times, making it particularly relevant for bTB studies. Furthermore, it considers genetic relationships through pedigree or genomic data and captures correlations between key epidemiological host traits such as infectivity and susceptibility. More recent versions of the SIRE software incorporate more complex epidemiological models (Prentice et al., 2022), such as an epidemiological SEIR model, previously proposed for bTB (Raphaka et al., 2018). By employing SIRE 2.0, researchers could estimate genetic parameters for all individuals involved in bTB breakdowns, overcoming the constraints of our index case approach. For example, it can generate EBVs for infectivity for all individuals, providing more accurate insights. Preliminary results also demonstrate the model's ability to handle various data complexities, such as irregular testing intervals and unmeasured infection times, while maintaining reliable accuracy in its predictions. To date, the SIRE tool has only been validated for simulated and experimental epidemics (Prentice et al., 2022; Pooley et al., 2024). Whilst the results are encouraging, application of this method to large scale national bTB field data would require significant further developments.

5.4.6 Incorporating infectivity into future breeding programmes

From our results, we see that bTB infectivity potentially has low genetic variance. Low genetic variance would lead to slow genetic progress, even when accurate estimated breeding values (EBVs) are available. To accelerate

progress under these constraints, future research could explore complementary approaches to improve selection strategies. For instance, focusing on identifying and managing high risk individuals or "super spreaders" may offer an effective alternative. Super spreaders contribute disproportionately to disease transmission, and targeting them for removal or reduced breeding weight could significantly reduce the overall transmission risk without requiring highly accurate estimates of infectivity for every individual (Lloyd-Smith et al., 2005). Additionally, selection index weights could be adjusted to place greater emphasis on infectivity traits, even if their genetic variance is small. For traits with low genetic variance, heavily selecting on these traits and integrating them carefully into selection indices could still drive meaningful reductions in bTB transmission. However, given the challenges of estimating infectivity accurately and the relatively small genetic variance observed, careful evaluation would be necessary to determine how best to implement these strategies into breeding programs. Future work may prioritize exploring and validating these approaches to optimize bTB control efforts within breeding programs.

5.5 Concluding Remarks

This thesis demonstrated the potential of genetic selection to reduce bTB transmission by identifying genetic variance in infectivity and utilizing models to estimate EBVs for this trait. These findings provided a promising foundation for selective breeding strategies aimed at mitigating disease spread. Despite these encouraging results, several challenges must be addressed before genetic selection for reduced bTB infectivity can be practically implemented. By addressing these gaps, the application of genetic selection could become a valuable addition to current bTB control strategies, offering long term benefits for disease management in cattle populations.

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