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Risk factors for reporting bovine tuberculosis-like lesions and confirmation of *Mycobacterium bovis* from bovine tuberculosis-like lesions from routine carcass inspection in cattle in New Zealand 2019 - 2021

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*This thesis is dedicated to my family, who largely released me from traditional domestic responsibilities allowing me the time to complete this project. The arduous transition back to adulthood lies ahead.*



## Abstract

**Aim:** In New Zealand, surveillance for bovine tuberculosis (bTB) is changing as the eradication programme matures. The transition from on-farm testing of livestock to predominantly slaughter surveillance for the detection of infection and providing evidence of disease absence has begun. The aim of this research is to provide data to support a critical evaluation of the effectiveness of slaughter surveillance for bTB in New Zealand. Specifically, the thesis explores risk-factors associated with i) bTB-like lesion submissions from cattle at routine carcass inspection and ii) confirmation of *Mycobacterium bovis* from bTB-like lesions submitted from cattle at routine carcass inspection.

**Methods:** Lifetime and bTB data were extracted from the National Animal Identification and Tracing database and Disease Management System database for cattle slaughtered for human consumption through registered meat processing plants between 1 January 2019 and 30 September 2021. The data sets were combined using the lifetime animal identification number and collated using R Statistical software (v4.1.2; R Core Team 2021). Animal-level data included sex, age, number of lifetime movements between farms, the bTB herd status of the herd where the animal was sent to slaughter from, the disease control area the animal was sent to slaughter from, the season and year of slaughter, and the meat processor identifier. Multivariable logistic modelling was used to identify risk-factors for bTB-like lesion submission from routine carcass inspection, and risk-factors for confirmation of bTB from bTB-like lesions submitted from routine carcass inspection.

**Results:** During the study period, there was one bTB-like lesion submitted for every 6378 cattle carcasses inspected. bTB was confirmed in one bTB-like lesion for every 60 bTB-like lesions submitted. During routine slaughter surveillance a bTB-like lesion was more likely to be submitted from an animal that came from an infected (OR 3.9, 95%CI 2.66 - 5.73) or suspended (OR 2.08, 95%CI 1.4 - 3.1) status herd, moved less than three times prior to slaughter (OR 1.17, 95%CI 1 - 1.27), and came from an area outside of movement control areas (OR 1.37, 95%CI 1.05 - 1.79). bTB was more likely to be confirmed from an animal that came from and infected status herd (OR 8.26, 95%CI 2.09 - 32.59), was female (OR 4.39, 95%CI 1.23 - 15.72), and came from a movement control area (OR 81.47, 95%CI 5.5 - 1192.37). The baseline risk of bTB-like lesion submission and confirmation of bTB differed between meat processing plants.

**Conclusion:** These findings indicate that meat processing plant-specific factors, not just animal-specific factors alone, influence whether a bTB-like lesion is submitted for testing or confirmed as bTB. Better understanding of factors influencing the probability for a meat processing plant to detect and submit bTB-like lesions would enable more tailored policy-making to improve the overall

submission rate. Identifying risk-factors for bTB-like lesion submissions and risk-factors for bTB-like lesions confirmed as bTB may identify enhancements to both on-farm surveillance, and a more efficient, risk-based approach to slaughter surveillance. The content of this research represents a first step for the overall evaluation of bTB slaughter surveillance as fit-for-purpose to support bTB eradication. A complete evaluation, based on this research, is warranted to determine required outputs and metrics for effective slaughter surveillance of bTB, and identify key areas for enhancement.

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## 1 Literature Review

## 1.1 Introduction

In New Zealand, bovine tuberculosis (bTB) in livestock has been actively managed since the 1950's, after its introduction with the early consignments of cattle in the 1800's (Laing, 1964; Smith, 2012). The Australian brushtail possum (*Trichosuris vulpecula*) plays a key role in the epidemiology of bTB in New Zealand, as a maintenance host and a vector for infection (Morris & Pfeiffer, 1995). By the mid-1990s, there were an estimated 1700 cattle and deer herds infected with bTB (Livingstone et al., 2015). To address the high level of bTB in livestock a co-ordinated, mandatory, government and levy funded Biosecurity (National Bovine Tuberculosis Pest Management Plan) Order 1998 was introduced affecting New Zealand's farming industry. Management and legal control of the pest management plan was assumed by the Animal Health Board, an industry-led advisory committee, whose mission was to eradicate bovine tuberculosis from New Zealand.

Animal health surveillance systems underpin successful disease eradication worldwide. In New Zealand, surveillance for bTB has evolved from a voluntary test and slaughter of animals positive to the tuberculin skin test, to compulsory testing of all cattle by 1975. The lack of reduction in infected herd numbers in response to test and slaughter in some parts of the country prompted research which confirmed possums were a wildlife vector for bTB (Ekdahl et al., 1970). The discovery of a wildlife vector for bTB lead to changes to the on-farm testing component of the surveillance system in areas where possums were known to be infected with bTB (Livingstone et al., 2015). Timely and relevant evaluations of the bTB surveillance systems in New Zealand has supported objective decision-making and provided for the efficient use of resources. These evaluations have enhanced acceptance of the surveillance system outputs by stakeholders.

In 2021, New Zealand's bTB surveillance system has begun a transition from on-farm testing to predominately inspection of carcasses at meat processing plants for signs of bTB as progress is made towards achieving bTB eradication. Inspection of carcasses at meat processing plants for signs of bTB will be referred to in this document as "slaughter surveillance". Evaluating the slaughter surveillance component of the bTB surveillance system in New Zealand will further support objective decision-making and aid in maintaining international trust and credibility of the bTB surveillance system. This chapter reviews animal health surveillance, provides a brief history of the purpose of bovine tuberculosis control in New Zealand, and reviews the literature on methods for evaluating animal health surveillance systems. Application of the principles and methods of evaluation to slaughter

surveillance for bTB in New Zealand will be discussed throughout in relation to the current objective of bTB eradication.

## 1.2 History of bovine tuberculosis pest management in New Zealand

The Biosecurity (National Bovine Tuberculosis Pest Management Plan) Order 1998 defines the purpose of bTB management in New Zealand. The principles of bTB management in New Zealand are: movement control of cattle and deer out of high-risk areas; livestock testing and within-herd management of bTB infection; and control of wildlife vectors to prevent the spread of disease. The bTB surveillance system consists of on-farm livestock testing and slaughter surveillance for the detection of bTB infected cattle and deer. The National Bovine Tuberculosis Pest Management Plan tests livestock on-farm using a risk-based system in which herds with the highest likelihood of acquiring infection from local wildlife vectors are tested at the highest frequency and require pre-movement testing. Herds where the risk of wildlife vector-related infection is believed to be low or non-existent are tested less frequently. The system also incorporated information about the number of years the herd had been either officially free from bTB infection, up to 10 years (C1-10), or infected with bTB. There have been four iterations of the National Bovine Tuberculosis Pest Management Plan since 1998, coinciding with changes in the purpose of bTB management which was supported by ongoing research. However, the principles of bTB management remained unchanged.

The purpose of the first National Bovine Tuberculosis Pest Management Plan (1998-2001) was to control the epidemic. Herds were assigned an alpha-numerical value indicating the number of years the herd had been either officially free from bTB infection, up to 10 years (C1-10), or infected with bTB. Livestock movement restrictions were applied to bTB infected herds and in areas where the risk of acquiring infection from wildlife was high. In addition to the livestock testing and movement restrictions, co-ordinated control of wildlife vectors was carried out to protect herds from infection with bTB. The combined approach of livestock management and vector control was effective at reducing the number of infected herds across New Zealand.

The purpose of the second National Bovine Tuberculosis Pest Management Plan (2002-2011) was to achieve and maintain official country bTB freedom. A country is said to achieve bTB freedom when the annual infected herd prevalence does not exceed 0.2% (World Organisation for Animal Health, 2021). At June 2012, the annual infected herd prevalence was 0.18% which was achieved through

test-and-slaughter and slaughter surveillance, livestock movement control, and a coordinated bTB-vector control programme.

The purpose of the third National Bovine Tuberculosis Pest Management Plan (2012-2015) was to determine if bTB eradication from possums and livestock was feasible. To determine the probability of bTB freedom in possums in a defined geographical area the Proof of Freedom Model, was developed. Outputs from the Proof of Freedom Model are used to objectively declare a possum population, within a defined geographical area, as free from bTB. The development and successful implementation of this framework has resulted in the Ministry for Primary Industry in New Zealand and other industry stakeholders to formally commit to a strategy for total biological eradication (Livingstone et al., 2015).

The purpose of the fourth, and current National Bovine Tuberculosis Pest Management Plan (2016-2055) is to achieve: freedom from bTB in livestock by 2026; freedom from bTB in possums by 2040; and total biological eradication by 2055. Freedom from bTB is defined by the statistical likelihood of bovine tuberculosis being present in the population of the species concerned not exceeding 0.0001% throughout the preceding 12-month period. The policy of total biological eradication relies on the fundamental assumption that slaughter surveillance can detect true bTB infection in cattle and deer if present, as on-farm testing is phased out. On-farm livestock testing is a costly component of a bTB surveillance system and for the year ending June 30<sup>th</sup> 2021, \$15 million was spent on livestock testing in New Zealand (OSPRI, 2021a). In 2021, New Zealand's bTB surveillance system began a transition from on-farm testing to predominately slaughter surveillance as progress is made towards achieving bTB eradication. Slaughter surveillance for bTB has replaced on-farm livestock testing in countries that have successfully eradicated bTB. These countries benefited from the economic efficiencies of a reduced intensity approach to on-farm livestock testing during the later stages of eradication as slaughter surveillance became the primary mechanism for detecting bTB (Napp et al., 2019; Sergeant et al., 2017).

### 1.3 Animal health surveillance

The World Organisation for Animal Health defines animal health surveillance as:

*“the systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information so that action can be taken”* (World Organisation for Animal Health, 2021, p. viii).

A surveillance system can consist of a single surveillance activity or contain multiple components, such as on-farm livestock testing and slaughter surveillance for the detection of bTB in New Zealand ("Biosecurity (National Bovine Tuberculosis Pest Management Plan) Order 1998, "). Common objectives of an animal health surveillance system include: case-finding to facilitate control; detecting new, emerging or exotic disease; substantiating claims of freedom from disease; describing the baseline level and distribution of disease; describing changes in the health of the population; describing changes in the population at risk; or improving the epidemiological understanding of disease (Drewe et al., 2015; Hoinville et al., 2013; World Organisation for Animal Health, 2021). The objective, or objectives of a specific animal health surveillance system can change over time. For example, the objective of Australia's bTB surveillance system transitioned from the identification of bTB cases to facilitate control, to providing evidence of disease absence in the late 1990s (Radunz, 2006). Despite the purpose of managing bTB in New Zealand changing over time, the objective of the surveillance system itself remains unchanged as case-finding to facilitate control. Total biological eradication is the current purpose of bTB management in New Zealand so the objective of the surveillance system will inherently shift to providing evidence of disease absence in the future. Demonstrating freedom from disease and case identification to facilitate control are the most common objectives of animal health surveillance systems worldwide (Bisdorff et al., 2017).

The data collected as part of surveillance activities may contribute to policy to mitigate the risk associated with animal diseases. Animal health surveillance systems can be used for: effective disease management; improving food systems productivity; providing assurance of food security, food safety, and animal welfare; and providing assurance to overseas markets, securing access to international trade (Peyre et al., 2019; Thiermann, 2015). Animal health surveillance is also recognised as playing an important role in predicting public health risks associated with zoonotic disease (George et al., 2020). The purpose of bTB surveillance in New Zealand is to enable effective disease management and provide assurance of food safety to local and overseas markets.

Routine meat inspection forms a component of the bTB surveillance system in countries worldwide and is the primary mechanism for substantiating freedom from disease in some countries (El Allaki et al., 2016; Ramanujam et al., 2021; Sergeant et al., 2017). The advantage of using routine meat inspection for disease surveillance is that it covers the population of animals slaughtered for human consumption and is inexpensive. The disadvantage of meat inspection for disease surveillance is that it may not meet the animal health authority's needs as there is little control over data quality (FAO, 2014). The lack of oversight of surveillance activities is particularly significant for surveillance programmes that are heavily or solely reliant upon slaughter surveillance for case detection or substantiating freedom from disease as cases may be missed.

Animal health surveillance systems can be input-based or output-based. Input-based surveillance systems are characterised by prescribed surveillance activities, in contrast to output-based systems that describe what surveillance must achieve (Cameron, 2012; Stark et al., 2006). Slaughter surveillance for bTB in New Zealand is an example of an input-based surveillance system. Under New Zealand legislation, during routine carcass inspection, meat inspectors are required to: palpate and incise lymph nodes where TB is commonly identified; view and palpate lungs and liver; and view visceral, pleural and peritoneal cavities (Ministry for Primary Industries, 2020). Although the primary function of meat inspection is to ensure food safety, inspectors are obligated to report the suspected presence of bTB. The requirement to report suspect bTB is the basis of the slaughter surveillance component of the bTB surveillance system ("Biosecurity Act," 1993). Output-based surveillance systems are driven by standards which are implemented for the purpose of achieving a desired surveillance system sensitivity, or to determine the probability of disease freedom of a population (Cameron, 2012). Determining what outputs are required from the system is essential to achieve surveillance objectives (More et al., 2015; USDA-APHIS, 2015).

Many countries have moved away from traditional input-based surveillance systems where surveillance activities are prescribed to output based systems that describe what surveillance must achieve (Cameron, 2012; Stark et al., 2006). In 2005, The United States Department of Agriculture established bTB-like lesion submission rate standards for Bison and Cattle slaughtered under Federal inspection to achieve a 95% confidence bTB would be detected at an animal prevalence of 0.0003% (USDA-APHIS, 2015). The same bTB-like lesion submission rate standards adopted by the United States Department of Agriculture to detect bTB at a prevalence of 0.0003% were implemented in Spain after the European Commission reviewed the progress of the Spanish program to eradicate bTB (Andreoletti et al., 2013). Australia also implemented similar bTB-lesion submission rate standards during the later stages of its eradication campaign (Animal Health Australia, 2007), although it is unclear what the surveillance standard was as there is no published methodology for how their submission rate targets were established.

Animal health surveillance systems can vary substantially, based on the epidemiology of a disease, but also economic factors, political instability, political strategy of a country, socio-economic context, and stakeholder requirements. Perception and expectations can influence the application, acceptability, and sustainability of animal health surveillance systems (Calba et al., 2016; Peyre et al., 2019). Farmer and veterinarian perception of diagnostic test performance and un-met expectations surrounding financial compensation when bTB is detected affects the acceptability of the bTB surveillance system in Belgium (Calba et al., 2016). Stakeholders' expectations of bTB management in New Zealand has changed with each iteration of the National Bovine Tuberculosis Pest

Management Plan. Implementation is, therefore, required to adapt to the evolving objectives and stakeholder expectations (Livingstone et al., 2015). Most commonly, countries rely on risk-based animal health surveillance, driven by the need for economic efficiency aligning with stakeholders expectations (FAO, 2014). Disease surveillance prioritisation tools have been developed to assist authorities when allocating funding to disease surveillance programmes that consider both likelihood and consequence of disease (Clarke et al., 2020).

## 1.4 Attributes of animal health surveillance systems

Attributes are the “many quantifiable characteristics of surveillance systems” (Drewe et al., 2015, p. 35). Frameworks to support and standardise animal health surveillance system evaluations focus on assessing these many quantifiable characteristics. The objectives of the surveillance system will determine the relevance of each attribute. For example, the relevance of timeliness will differ between a surveillance system to detect a fast-moving exotic disease and one to detect new cases of a chronic endemic disease. Some frameworks are prescriptive and rigid with what attributes are included in an evaluation (Hendriks et al., 2011), while others offer flexibility and provide decision support tools for prioritising attributes based on relevance to the surveillance objectives, such as the SERVAL framework (Drewe et al., 2015; Muellner et al., 2018).

An evaluation of slaughter surveillance for bTB in New Zealand should include an assessment of attributes of importance to the current objective of surveillance as well as attributes of importance to the intended future objective of the system. Evaluating attributes of importance to the intended objective of the surveillance system would provide value by pre-emptively identifying possible efficiencies. Attributes of primary importance to case-finding to facilitate control or demonstrate freedom from infection include sensitivity, specificity, benefit, communication, cost, coverage, participation, representativeness, and timeliness. The current section of the literature review explores the nine attributes of slaughter surveillance for the detection of bTB and their significance in the New Zealand context.

### 1.4.1 Sensitivity

The sensitivity of a surveillance system can be considered on three levels including: case detection, referring to individual animals or herds that have the condition of interest that the surveillance system is designed to detect; outbreak detection, referring to the probability that the surveillance system will detect a pre-defined increase of disease incidence; and presence, referring to the

probability that disease will be detected at a certain prevalence in the population (Drewe et al., 2012; Hoinville et al., 2013).

Slaughter surveillance for the detection of bTB is well documented in the literature as having a low and variable sensitivity which can relate to several factors (Corner et al., 1990; Downs et al., 2018; Frankena et al., 2007; Nunez-Garcia et al., 2018; Olea-Popelka et al., 2012). The factors influencing the sensitivity of slaughter surveillance for bTB include the probability that a bTB infected animal has grossly detectable lesions, the probability that a lesion is detected at routine carcass inspection, the probability that bTB lesions are submitted for diagnostic testing, and the sensitivity of diagnostic tests used for confirmation (El Allaki et al., 2016; Garcia-Saenz et al., 2015; Sergeant et al., 2017). A report on the sensitivity of slaughter surveillance for bTB in New Zealand stated that 81.4% of bTB infected animals were detected at routine meat inspection in New Zealand (Nugent, 2017). The estimated sensitivity of slaughter surveillance in New Zealand is likely to be an overestimate as the meat inspectors were aware the animals from which data were collected were part of a vaccination trial risking observer bias. In addition, the within-herd prevalence was high and the slaughterhouse processing the animals serviced a region with a long-standing high prevalence of bTB (suggesting that the meat inspectors were experienced). The estimate of the sensitivity of slaughter surveillance for bTB in New Zealand provided by this report cannot be extrapolated to the population of meat processing plants. The sensitivity of post-mortem diagnosis of bTB in New Zealand had previously been reported at 50% (Pociecha Jz, 1990). A meta-analysis of post-mortem diagnostic tests for bTB estimates the sensitivity of routine carcass inspection for the detection of bTB to be 71% (95% CI 0.37-0.92)(Nunez-Garcia et al., 2018).

The sensitivity of a surveillance system will be reduced when the disease of interest is grossly indistinguishable from other conditions (FAO, 2014; Thursfield & Christley, 2018). The distinctive feature of tuberculosis in cattle is the presence of granulomatous inflammation (World Organisation for Animal Health, 2021). However, the ability to visually differentiate between granulomatous lesions caused by tuberculosis, *Actinobacillus*, *Rhodococcus*, and *Staphylococcus* at post-mortem inspection is difficult (Corner et al., 1990; Pociecha Jz, 1990).

Output-based surveillance programmes to enhance the sensitivity of slaughter surveillance for the detection of bTB have been implemented by Australia, Spain and the USA (Sergeant et al., 2017; USDA-APHIS, 2015). Slaughter surveillance policies were based on pre-defined target rates of bTB-like lesion submissions for confirmation. Increasing submission rates of bTB-like lesions was achieved by establishing national granuloma submission rate targets, incentivising and rewarding performance of meat inspection teams, and communicating regularly with those involved in

implementation of the surveillance system (More et al., 2015). During the implementation of policies to enhance the sensitivity of slaughter surveillance for bTB, key constraints to achieving targets included low levels of system user motivation; meat inspector and veterinary officers' lack of experience; time constraints; line management; lack of feedback for efforts expended; and unknown slaughter premise-related factors (Frankena et al., 2007; More & Roe, 2002; Olea-Popelka et al., 2012; Pascual-Linaza et al., 2017; Pozo et al., 2021). The disadvantage of this approach to increase the sensitivity is a decrease in specificity.

#### 1.4.2 Specificity

Specificity refers to the number of true non-cases classified as such. The inverse of this would be the false alarm rate (Drewe et al., 2012; Hoinville et al., 2013). Considering bTB is grossly indistinguishable from other conditions at post mortem inspection (FAO, 2014; Thursfield & Christley, 2018), the expectation is that carcass inspection will produce false positive results for the detection of bTB lesions. Therefore bTB-like lesions are sent for confirmatory tests before a determination of bTB infection is made (OSPRI, 2021b). The specificity of post-mortem diagnosis of bTB in New Zealand had previously been reported at 89% (Pociecha Jz, 1990).

The cost of a high specificity for bTB detection at routine carcass inspection may be a lowered sensitivity as true cases of bTB go undetected. The consequence of decreasing the specificity of meat inspection for bTB to improve sensitivity will vary between countries and even between slaughterhouses. The disposition of a carcass with bTB like lesions has overseas market access implications and can affect farmer payment premiums. Decreased specificity is likely to result in an increased cost to the management agency through additional sample transport costs and laboratory diagnostics fees. The objective of the surveillance system will influence whether the increased cost of lowered specificity is worth the increased sensitivity. Hence quantifying the unintended consequences of changing the desired output of the specificity attribute would be paramount.

#### 1.4.3 Benefit

The benefit of a surveillance system refers to the direct and indirect advantages of a surveillance system (Drewe et al., 2012; Hoinville et al., 2013). The benefit of bTB management is difficult to quantify considering its zoonotic potential, perceived benefits to public health and its requirement to secure international market access (World Organisation for Animal Health, 2021). The perceived benefit of bTB management will differ between countries according to their socio-economic status and trade partnership agreements. Some authors argue any benefit of bTB management in high-income countries and challenge the justification for expenditure (Caminiti et al., 2016).

The primary motivation for developing risk-based animal health surveillance systems is to benefit from economic efficiencies when disease incidence is low or when the objective of the surveillance system is inefficiently achieved using a conventional approach (Calba et al., 2015; Drewe et al., 2012; Hernandez-Jover et al., 2021; Stark, 2012; Stark et al., 2006). The information that slaughter surveillance for bTB provides is considered valuable and low cost (FAO, 2014). However, economic evaluations of surveillance systems are rarely carried out. Economic evaluation of animal health surveillance systems for diseases with public health significance is technically challenging because of the complex data requirements and the dynamic relationship between surveillance, intervention, loss avoidance and non-monetary benefits. Difficulty quantifying the interactions between these relationships is compounded by the lack of standardised metrics and tools for economic appraisals (FAO, 2014; Peyre et al., 2019).

In New Zealand, the benefit of managing bTB is maintaining access to international markets through demonstrating a commitment to animal health (OSPRI, 2021a). The benefit of slaughter surveillance for bTB is poorly described in the literature. The benefit of slaughter surveillance for bTB in New Zealand is likely consumer and stakeholder perception and economic efficiencies over the benefit to public health specifically.

#### 1.4.4 Participation

Participation refers to the extent to which people involved in the implementation of the surveillance system engage with the surveillance process (Drewe et al., 2012; Hoinville et al., 2013). Describing how different user groups interact with the surveillance system has the benefit of identifying aspects of the surveillance system where administrative or human resource factors may be impacting implementation of bTB management policies. User groups for bTB surveillance include meat inspectors, verification veterinarians, bTB testers and laboratory technicians. For slaughter surveillance for bTB, participation has been measured by key indicators including submission rates and confirmation rates of bTB-like lesions. Monitoring these indicators and providing feedback to system users on progress towards achieving predetermined standards has been encouraged by policy makers (Veterinary and International Affairs, 2013).

Reported granuloma submission rates for bTB confirmation vary significantly between countries from one lesion per 192 animals slaughtered in Ireland (Olea-Popelka et al., 2012), to one lesion per 6400 animals slaughtered in Great Britain (Shittu et al., 2013) and one lesion per 20,000 animals slaughtered in Spain (Pozo et al., 2021). Granuloma submission rate variation between meat processing premises also varies significantly and has been attributed to slaughter-premise factors, rather than animal-specific risk-factors (Frankena et al., 2007; McKinley et al., 2018; Pascual-Linaza et al., 2017; Pozo et al., 2021; Radunz, 2006).

Australia, Spain and the USA established submission rate targets in order to achieve a desired confidence of freedom from disease and applied strategies such as having designated officers to train, motivate and communicate with system users to maximise participation (Poza et al., 2021; Sergeant et al., 2017; USDA-APHIS, 2015).

#### 1.4.5 Communication

Communication refers to the methods and ease of which information is exchanged between people involved in the surveillance system (Drewe et al., 2012; Hoinville et al., 2013). Communication with those interacting with a surveillance system has been identified as a factor that influences co-operation which can impact on surveillance efforts (Thursfield & Christley, 2018). Ensuring the objectives of surveillance are communicated to all involved and providing feedback to match the efforts expended has been encouraged to maintain engagement with systems users (More & Roe, 2002). The USA, Spain and Australia implemented granuloma submission rate targets as a feature of the slaughter surveillance component of their bTB surveillance system. Reports on slaughter surveillance programmes for the detection of bTB describe roles that were established to maintain engagement, monitor compliance, conduct site visits, deliver workshops and provide training, and keep system users informed of progress towards achieving targets (More & Roe, 2002; USDA-APHIS, 2015). In New Zealand, assessing the relationship between the meat inspection agency, state verification veterinarians and the bTB management agency would be essential for evaluating communication as a surveillance system attribute.

#### 1.4.6 Cost

Cost as an attribute of a surveillance system refers to the tangible cost of surveillance and can be itemised (Drewe et al., 2012; Hoinville et al., 2013). Evaluation of costs associated with surveillance systems often focuses on the direct cost of on-farm testing and laboratory services, and costs incurred by farmers. Kao et al. (2018) modelled the cost-effectiveness of several alternative risk-based bTB surveillance systems in Minnesota. The costs were relative to the existing slaughter surveillance-only system and considered direct costs only. In New Zealand, the costs incurred by the bTB management agency would include sample transport costs and laboratory diagnostic fees. Costs incurred by farmers and meat processing premises include the direct cost associated with condemnation of carcasses, carcass exclusion from export market, and the indirect cost of the risk of occupational exposure of meat inspectors to a zoonotic disease during handling and collection of samples for confirmation which would be difficult to quantify (Elmonir & Ramadan, 2016).

#### 1.4.7 Coverage

Coverage refers to the proportion of the population of interested covered by the surveillance activity (Drewe et al., 2012; Hoinville et al., 2013). Slaughter surveillance for bTB is considered passive

surveillance considering that the primary role of carcass inspection is food safety. All cattle slaughtered commercially for human consumption are subject to routine carcass inspection where the proportion of the population of interest subject to that activity is 100%. Coverage is excellent in principle. The proportion of the national cattle population being slaughtered is dynamic and poorly described in the literature. In a surveillance system where the combined activities of on-farm testing and slaughter surveillance contribute to the overall sensitivity of the surveillance system, a better understanding of the population of cattle slaughtered would be of value. Several simulation models have utilised slaughter demographic data in simulating bTB control programme options suggesting the data and means of evaluation are available in some countries (Napp et al., 2019; Willeberg et al., 2018).

#### 1.4.8 Representativeness

Representativeness refers to the extent to which features of the population of interest are reflected in the surveillance data collected (Drewe et al., 2012; Hoinville et al., 2013). Determining what features of the population of interest are important to bTB management should proceed an evaluation of representativeness. Considering the geographical risk of acquiring bTB infection from contact with infected wildlife in New Zealand, geographical representativeness would be an important feature to evaluate. In theory, coverage of the surveillance system is excellent, but participation may differ between meat processing plants which may impact on representativeness. Network analyses have been carried out on livestock bTB testing models (Cardenas et al., 2021) but not to assess representativeness at slaughter inspection specifically. The National Animal Identification and Tracing database in New Zealand which records lifetime location information holds data which would allow for an analysis of representativeness.

#### 1.4.9 Timeliness

Timeliness is a measure of time between any two defined steps in a surveillance system (Drewe et al., 2012; Hoinville et al., 2013). Timeliness will be influenced by what diagnostics are used within the surveillance system (Nunez-Garcia et al., 2018), maximum reporting timeframes specific to individual contractors involved with the surveillance system, or compliance with relevant legislative requirements for reporting ("Biosecurity (National Bovine Tuberculosis Pest Management Plan) Order 1998,"). Time to detection as an outcome variable has been included in simulation modelling of bTB control options (Napp et al., 2019), but no evaluations of timeliness have been carried out for slaughter surveillance for bTB.

## 1.5 Evaluation of Animal Health Surveillance Systems

Timely and relevant evaluations of animal health surveillance systems aid in establishing and maintaining international trust and credibility; supporting objective decision-making; providing for the efficient use of resources; and enhancing acceptance of the surveillance system outputs by stakeholders (Drewe et al., 2015; FAO, 2014; Peyre et al., 2019). Evaluations can be carried out on the whole, or just a component of a surveillance system. The technical performance of the system, or the processes contributing to the overall operation of the system can be evaluated. It can also be a comparative evaluation with alternative systems, to draw lessons from past programmes and propose improvements in implementation. Despite their importance, comprehensive evaluations of animal health surveillance systems are rarely carried out (George et al., 2020). Drewe et al. (2012) evaluated 99 published articles evaluating surveillance systems. Only 50% of these articles evaluated more than two attributes.

### 1.5.1 Animal health surveillance system evaluation frameworks

Animal health surveillance systems now vary widely in their methods. The need for a comprehensive and flexible method for standard evaluation has thus long been recognised by researchers and policy makers. The technical challenges of designing and evaluating these unique systems has prompted the development of frameworks that serve as a set of dedicated suitable methods and tools, to support policy makers and veterinarians for standard evaluations (Drewe et al., 2015; Muellner et al., 2018; Peyre et al., 2019). A gold-standard evaluation framework would facilitate a complete evaluation considering epidemiological, sociological, and economical aspects (Peyre et al., 2019). It would be flexible to allow for different surveillance system objectives, constraints and resourcing for the evaluation as well provide methods and tools for selecting and assessing relevant attributes for evaluation (Calba et al., 2015; Drewe et al., 2015; Drewe et al., 2012; Hendrikx et al., 2011; Muellner et al., 2018; Peyre et al., 2019).

Most high-income countries (e.g. United States of America, United Kingdom, New Zealand, France) list the ability to assess the quality and improve efficiency of surveillance as a priority in their respective surveillance and biosecurity strategic plans. The commitment to improving surveillance efficiency motivated the development of generic frameworks to standardise the evaluation of their biosecurity portfolios (Drewe et al., 2015; Hendrikx et al., 2011; Muellner et al., 2018). The European Union funded a consortium, the RISKSUR project, to investigate novel approaches for cost-effective surveillance integrating advances in epidemiological methodologies and produced an evaluation tool to complement their surveillance system design tool (Peyre et al., 2019).

In addition to developing tools to support government's strategic priorities, another common motivation for the development of new animal health surveillance system evaluation frameworks is to address the inadequacies of existing frameworks (Calba et al., 2015; Drewe et al., 2015; Hendriks et al., 2011). The primary inadequacy of existing frameworks is the lack of flexibility to allow for evaluations of such a wide variety of animal health surveillance systems across different species and socio-economic contexts. A lack of clear guidance and provision of support tools for conducting the evaluation and economic analysis was also a strong motivator for developing new frameworks (Comin et al., 2016; Drewe et al., 2015; Peyre et al., 2019). New frameworks are typically developed by building on existing frameworks from both public and animal health disciplines, by selecting and modifying features of existing frameworks to align with the evaluator's needs.

Animal health surveillance evaluation frameworks have been developed using an interdisciplinary approach (Calba et al., 2015). Veterinarians, epidemiologists, and economists work in close collaboration with stakeholders representing an academia, government, and industry partnership. The methodology for developing the frameworks included reviewing available literature for existing frameworks and methods of evaluations in practice, identifying the specific needs of the evaluation framework developers, and producing a corresponding framework that was fit-for-purpose. These steps are carried out through workshops and regular face-to-face meetings. In addition, case-study evaluations can be carried out to demonstrate and discuss the framework from an operational perspective (Drewe et al., 2015; El Allaki et al., 2013; Hendriks et al., 2011; Muellner et al., 2018; Peyre et al., 2019). A period of consultation can be followed by refinements to the framework. The development of these frameworks can take several months to several years to complete.

#### *1.1.1.1 Application of animal health surveillance system evaluation frameworks*

Animal health surveillance system evaluation frameworks are structured to assist the evaluator define the motivation for and the scope of the evaluation; characterise the surveillance system under evaluation; design and conduct the evaluation; and communicate evaluation outputs. Frameworks are designed to accommodate a variety of different methodologies and provide tools to support framework users to complete the task. The usability of the frameworks that have been developed for evaluating animal health surveillance systems varies. Their application ranges from being limited to skilled users of specific software and those with advanced surveillance skills and knowledge (Hendriks et al., 2011; Peyre et al., 2019), to being appropriate for "anyone familiar with epidemiological concepts and with a reasonable knowledge of the disease under surveillance" (Drewe et al., 2015, p. 39). The framework developed by El Allaki et al. (2013) is the only one that allows for the evaluation of theoretical surveillance systems before they become operational. Other

frameworks available are performance-based, meaning that only functioning surveillance systems can be evaluated.

Common supporting material provided by developers of frameworks for evaluating animal health surveillance systems include a glossary of terms, attribute definitions, guidance notes on how to use the framework, a description of methods and published examples of how attributes have been evaluated, and case studies of the framework in use (Drewe et al., 2015; Hendriks et al., 2011; Muellner et al., 2018; Peyre et al., 2019). Some frameworks offer simple questionnaires, scoring guides, lists of assessment criteria, downloadable templates and excel spreadsheets for generating visual representations of evaluation outputs (Hendriks et al., 2011; Muellner et al., 2018). Some frameworks provide decision support tools, including attribute prioritisation matrices and decision-tree pathways, to help form evaluation questions and to define evaluation priorities (Drewe et al., 2015; Peyre et al., 2019). The more modern and comprehensive frameworks that were developed recently provide an online platform for carrying out the evaluation, calculation tools, questionnaires, detailed economic evaluation methods, and comprehensive guidance notes with links to other online support material (Peyre et al., 2019). Methods by which surveillance system attributes can be evaluated include quantitative, qualitative or a combination approach. The Centre for Disease Control and Prevention (USA) Guidelines for Evaluation of Public Health Surveillance Systems prescribe the use of both quantitative and qualitative methods to ensure more accurate results.

Once an evaluation of an animal health surveillance system has been completed, communicating findings with stakeholders and policy makers facilitates the transfer of knowledge required for operational changes to be considered. In one example of a more prescriptive framework, an excel spreadsheet was provided to generate and populate histograms, bar charts and radar charts to describe attribute performance (Hendriks et al., 2011). Other frameworks use traffic light systems to subjectively summarise appraisals of selected attributes (Drewe et al., 2015; Muellner et al., 2018). Many frameworks provide generic recommendations regarding appropriate methods of communication for specific target audiences while others provide detailed roadmaps on how to report evaluation outputs to decision makers (Peyre et al., 2019).

## 1.6 Conclusion

bTB management in New Zealand is evolving and the importance of slaughter surveillance is increasing relative to on-farm testing. This shift is consistent with global trends in bTB management during the later stages of bTB eradication programmes. Currently there is no published work to

critically evaluate slaughter surveillance in New Zealand for the detection of bTB and its suitability to compliment or supplement a diminishing on-farm livestock testing program. It is therefore timely that a review of means of evaluating animal health surveillance systems be undertaken to underpin an evaluation of slaughter surveillance for bTB in New Zealand.

There are several attributes of animal health surveillance systems and their relevance to evaluating slaughter surveillance for bTB are reviewed. Evaluating attributes essential to achieving disease management objectives such as sensitivity and representativeness, alongside attributes where collaboration between all agencies involved, such as participation and communication, would provide a good foundation for an evaluation of slaughter surveillance for bTB in New Zealand. Having reviewed the literature on evaluation of animal health surveillance systems through the lens of slaughter surveillance for bTB in New Zealand, the following chapters are intended to provide research to support a complete evaluation of the slaughter surveillance component of the bTB surveillance system in New Zealand.

## 2 Risk factors for bovine tuberculosis-like lesions reported at routine carcass inspection in cattle in New Zealand

## 2.1 Introduction

In New Zealand, bovine tuberculosis (bTB) in livestock has been actively managed since the 1950's, after its introduction with the early consignments of cattle in the 1800's (Laing, 1964; Smith, 2012). The Australian brushtail possum (*Trichosuris vulpecula*) plays a key role in the epidemiology of bTB in New Zealand as a maintenance host and a vector for infection (Morris & Pfeiffer, 1995). By the mid-1990's, there were an estimated 1700 cattle and deer herds infected with bTB (Livingstone et al., 2015). In 1998, a co-ordinated, mandatory, government- and levy-funded Biosecurity (National Bovine Tuberculosis Pest Management Plan) Order 1998 was introduced to address the bTB epidemic affecting New Zealand's farming industry.

In New Zealand, the bTB surveillance system consists of on-farm livestock testing and slaughter surveillance for the detection of bTB cases. Under New Zealand legislation, during routine carcass inspection, meat inspectors are required to palpate and incise the lymph nodes where bTB is commonly identified, and report suspect bTB (Biosecurity (National Bovine Tuberculosis Pest Management Plan) Order 1998). The requirement to report suspect bTB is the basis of the slaughter surveillance component of the bTB surveillance system. New Zealand is progressing towards bTB eradication with a view of achieving bTB freedom from livestock by 2026, bTB freedom from the wildlife vector by 2040, and total biological eradication by 2055.

Routine carcass inspection forms a component of the bTB surveillance system in countries worldwide and is the primary mechanism for substantiating freedom from disease in some countries (El Allaki et al., 2016; Ramanujam et al., 2021; Sergeant et al., 2017). Livestock testing is often implemented using a risk-based approach. In New Zealand, risk-based testing of livestock is based on risk areas where there is known bTB infection in the wildlife vector. However, total eradication usually relies on slaughter surveillance rather than on-farm livestock testing in the later stage of the process (Napp et al., 2019; Sergeant et al., 2017).

The sensitivity and specificity of slaughter surveillance for bTB requires evaluation. Nugent (2017) and Pocięcha Jz (1990) have reported the sensitivity and specificity of slaughter surveillance at 81% and 89%, respectively. Since then, there has been a reduction in the proportion of newly-infected herds in New Zealand identified through routine carcass inspection (OSPRI database). The sharp decrease, in the absence of significant changes to the routine on-farm testing policy may signify a reduction in the ability of carcass inspection to detect a bTB animal, that is there has been a reduction in the sensitivity of carcass inspection. Therefore, there is a need to evaluate the slaughter surveillance component of the surveillance system and its suitability to support the late stage of the

eradication process. The aim of this study is to assess risk-factors associated with bTB-like lesion submissions from cattle at routine carcass inspection to evaluate the effectiveness of current slaughter surveillance practice to underpin a transition from on-farm testing to predominantly slaughter surveillance.

## 2.2 Materials and methods

The study population was all cattle slaughtered through eligible meat processing plants that were subject to routine carcass inspection between 1 January 2019 and 30 September 2021 in New Zealand. There were 53 registered meat processing plants that slaughtered cattle between 1 January 2019 and 30 September 2021 in New Zealand. Meat processing plants where more than 10 cattle were slaughtered for human consumption during the study period were included in the analysis. A total of 44 meat processing plants met the inclusion criteria and were included in the analysis. Two petfood processing plants and seven small premises did not meet the inclusion criteria. A total of 7856 cattle (0.1% of the total population) were processed through the nine meat processing premises that were excluded from the analysis. Another 757 reactor cattle (cattle positive to one or more ante-mortem bTB tests) processed through 13 of the 44 eligible meat processing plants were also excluded from the analysis on the basis reactor cattle were not subject to routine carcass inspection, resulting in a total of 7,646,655 inspected carcasses in the study population. When a bTB-like lesion was reported during routine carcass inspection, the lesion was sampled, and fresh and fixed specimens were prepared. The samples were sent to one commercial veterinary laboratory for histopathological examination. Animal details from routine carcass inspection were entered into the Disease Management System (DMS) database for cattle with bTB-like lesions reported and submitted. The outcome variable is a binary variable coded as 1 for “bTB-like lesion submitted” and 0 for “No bTB-like lesion submitted”.

Herd level data and accompanying individual animal bTB data was extracted from DMS for cattle with bTB-like lesions submitted during the study period. Animal-level data was extracted from the National Animal Identification and Tracing (NAIT) database for all cattle slaughtered during the study period, and included sex, age, number of lifetime movements between farms, the bTB herd status of the herd where the animal was sent to slaughter from, the disease control area the animal was sent to slaughter from, the season and year of slaughter, and the meat processor identifier.

The data sets were combined using the lifetime animal identification number and collated using R Statistical software (v4.1.2; R Core Team 2021).

## 2.2.1 Description of explanatory variables

### 2.2.1.1 *Disease control area*

In New Zealand, bTB management varies according to the risk of acquiring bTB from the wildlife vector. These areas are referred to as disease control areas and are presented in Figure 2-1. There are four disease control areas. In order of highest risk to lowest risk the area risk categories are: 1. Movement control area, 2. Special testing area annual, 3. Special testing area biennial, 4. Surveillance testing area. Movement control areas are defined geographical areas where infected herd annual period prevalence is greater than one percent or at the discretion of the veterinarian based on knowledge of other risk-factors such as history of wildlife vector control and presence of wildlife infection (OSPRI, 2021b). Special testing areas serve the purpose of providing additional bTB surveillance, mainly to detect the presence of infected vector populations. Surveillance testing areas constitute the remaining balance of New Zealand's land area outside of movement control areas and special testing areas and are free of known infected wildlife vectors.

Changes to the disease control areas occur annually following the proof of freedom process where geographical areas are objectively declared as free from bTB (OSPRI, 2021b). Disease control area changes are made relative to risk of bTB infection from wildlife both in time and space.

For analyses, we used the disease control area corresponding to the last location of the cattle before slaughter. For animals sent to slaughter from a saleyard, the disease control area of the location before the last location before slaughter was used. For comparison, we also evaluated the similarity in the risk level of the disease control area for the last location before slaughter versus the one before last location versus the birth location, in the general population, in animals sent to slaughter from saleyards, and in animals that stayed less than 30 days at their last location before slaughter (Table 2-3). The 30-day cut-off was based on the minimum time required for bTB-like lesions to develop in experimentally infected cattle (Cassidy et al., 1998; Palmer et al., 1999).

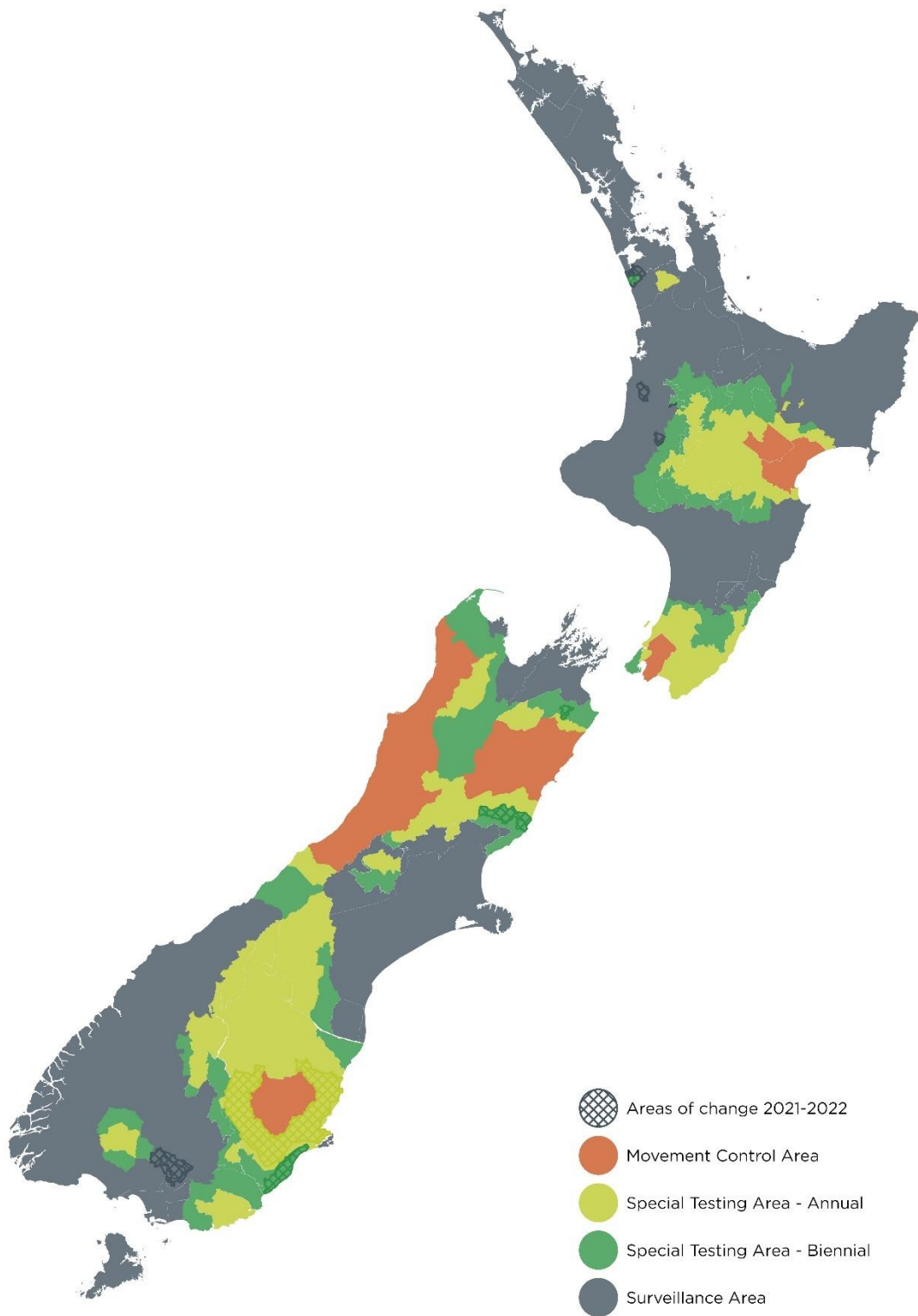


Figure 2-1 Geographical representation of New Zealand bovine tuberculosis disease control areas as of 01/02/2022. Map taken from the OSPRI Annual Report 2021 – 2022 (OSPRI, 2022).

#### 2.2.1.2 Age

Age at slaughter was determined by the difference in days between date of birth and date of slaughter. Date of birth was known for 41.7% of the population slaughtered. For animals with a known date of birth, the median difference in days between birth date and registration within the NAIT database date was 40 days (3<sup>rd</sup> quartile 183 days). The median age at death for animals with a known date of birth was 962 days (Inter quartile range 772 to 1685 days). Farmers are required to register an animal in the NAIT database within 180 of birth. The median age at death for animals where birth date was unknown and registration date was used as a proxy for date of birth was 886 days (Inter quartile range 576 to 1737 days). Date of registration was considered an acceptable proxy for date of birth and was used for 22.7% of the study population. The decision was made to categorise age using a biologically plausible cut off. The median age at slaughter was 3 years. Age was categorized into <3 years and ≥3 years.

Birth date and registration date were unknown for 37.3% of the study population. These animals were “auto-registered” in the NAIT database when a movement was first created for them. Animals for which the time between auto-registration and date of slaughter was over 1095 days (n=132,058) were included in the ≥3 age category. The remainder had unknown age (35.6% of total population slaughtered). The high proportion of missing age precluded the use of age in the multivariable model, but we still describe age in the descriptive results.

#### 2.2.1.3 Production type

Production type is a compulsory field in the NAIT database (Dairy or Beef). Slaughter levies for the bTB programme and the NAIT programme are collected based on production type, hence there was a very high level of data completion for this variable (only 1.7% of unknown production type). Production type is declared upon registration in the NAIT system but can be changed during the lifetime of the animal.

#### 2.2.1.4 Sex

Sex (male or female) is a voluntary field in the NAIT database. When an animal with a bTB-like lesion was reported at routine carcass inspection, necropsy and animal details were entered into DMS database. Sex was not considered for multivariate analysis due to the high percentage of missing data (84.2%), but we still describe sex in the descriptive results.

#### 2.2.1.5 Herd status

Herd status is categorised in the DMS database as follows. Breeding herds free from known bTB infection are categorised as C (clear), followed by a number (1 to 10) representing the number of years the herd has been officially free from bTB. These herds are subject to routine on-farm testing specific to their area testing requirements (OSPRI, 2021b). Beef production herds are assigned a CM

(clear monitored) status and not subjected to regular routine on-farm testing. The clear TB status of CM herds is maintained through slaughter surveillance. Infected herds are categorised as 'I', followed by a number representing the number of consecutive years the herd has been official infected with bTB. An 'S' was used to represent a suspended status of a newly formed herd, a herd where a clear bTB status has been suspended pending diagnostic results on a bTB suspect animal, a herd non-compliant with the bTB programme policy, or other epidemiological or risk-based assessment.

When an animal is sent to slaughter, a movement is generated in the NAIT database from the NAIT location the animal was sent to slaughter from to the meat processing plant. The bTB herd status the animal was sent to slaughter from is not recorded in the NAIT database and was not available within the NAIT data. There was not a 1:1 relationship between herd number and NAIT location.

The herd status was extracted from the DMS database and merged by herd number with the NAIT animal-level dataset of slaughtered animals when there was a perfect match by herd number and NAIT number in both databases. For a subset of the data, the herd number was missing in the NAIT database, but if the NAIT number was a match in the DMS database the herd status was used. In some cases, there were up to nine herd numbers and therefore herd status' per NAIT number in the NAIT database. If there were different herd status' present at one NAIT number, the highest risk was attributed to the corresponding NAIT location (i.e. Clear if all the herds present at a NAIT location were clear herds, suspended if there was at least one herd with a suspended status, and infected if there was at least one herd with an infected status present at the NAIT location).

#### *2.2.1.6 Number of lifetime movements*

Farmers have a legal obligation to record all animal movements into the NAIT database. Lifetime animal movements was considered a proxy for contact with other livestock. Number of movements were categorized into  $\leq 2$  movements or  $> 2$  movements.

#### *2.2.1.7 Temporal variable*

Season and year were both considered for inclusion. The dataset did not cover three calendar years and to avoid drawing conclusions based on a comparison of incomplete seasons and years, a variable that captured both the year and season was created. Summer months included December, January, and February. Autumn months included March, April, and May. Winter months included June, July, and August. Spring months included September, October, and November.

### 2.2.2 Data analysis

Data analysis was performed using R (v4.1.2; R Core Team 2021). Continuous data were assessed for normality using histograms. Non-normally distributed continuous variables were converted to a categorical variable using the quartiles. For categorical variables, the Pearson's Chi-squared test of proportions was used to compare the risk of bTB-like lesion submission between levels. Categorical variable levels were collapsed if there were no statistically significant difference between them using a p-value of  $<0.05$ , and if it was biologically appropriate.

A bivariate logistic regression model was carried out for all explanatory variables, using the submission of a suspect bTB lesion following routine carcass inspection (0 or 1) as the outcome. Results were presented as odds ratio and the 95% confidence interval. All explanatory variables with a likelihood ratio chi-square test p-value of  $<0.2$  were included in the multivariable model building.

Multivariable analyses were performed using a manual backwards elimination procedure to obtain a parsimonious model. Meat processing plant was used as a random effect in the model to account for clustering of the outcome within plants and evaluate variation in the outcome between meat processing plants.

Collinearity between explanatory variables was assessed visually during data exploration for variables where collinearity may exist, but no obvious issue was detected. Collinearity between explanatory variables included in the final model was further evaluated using the variance inflation factor. All explanatory variable variance inflation values were less than five so collinearity was not considered an issue.

## 2.3 Results

There were 7,646,655 cattle that were subject to routine carcass inspection at the 44 meat processing plants included in the study between 1 January 2019 and 30 September 2021. Of these, a total of 1199 bTB-like lesions were reported at routine carcass inspection and submitted to the veterinary laboratory for diagnostic testing, by 31 meat processing plants, representing an overall submission rate of one bTB-like lesion for every 6378 carcasses inspected. Numbers and percentages of lesions submitted for each category of the explanatory variables are presented in Table 2-1.

The percentage of population slaughtered, and percentage of bTB-like lesions submitted by meat processing plant are displayed in Figure 2-2.

Submission rates by meat processing plant are presented in Table 2-2 Number of cattle slaughtered per bovine tuberculosis-like lesion submitted with 95% confidence intervals from 44 meat processing plants that submitted 1199 bovine tuberculosis-like lesions from 7,646,655 cattle at routine carcass inspection between 1/1/2019 and 30/9/2021. There was a statistically significant difference between bovine tuberculosis-like lesion submission rates by meat processing plant using generalised linear modelling with a likelihood ratio test p-value of <0.001. and are described as the number of cattle slaughtered per bTB-like lesion submitted. There were 13 meat processing plants that submitted no bTB-like lesions, while one plant submitted 777 lesions representing 65% of the total number of lesions submitted.

Results of the bivariate analysis are presented in Table 2-4. All explanatory variables had a p-value of <0.2 and were included in the multivariable model building. Sex and Age were excluded from the multivariable analysis due to missing data.

The distribution of random intercepts for meat processing plants is displayed in Figure 2-3. This represents the variation per plant of the baseline odds of bTB-like lesion submission at routine carcass inspection, at fixed levels of all other model covariates.

The disease control area an animal was sent to slaughter from in relation to that of the one before last location and that of their birth disease control area is summarised in Table 2-3. The median time an animal spent at the location they were sent to slaughter from was 443 days (1<sup>st</sup> quartile 202 days; range 1 to 3704 days).

The outcome (bTB-like lesion submitted) was clustered by herd of origin. Using the NAIT location as a proxy for herd of origin, the 1199 lesions came from 749 NAIT locations, each having submitted between one and 24 lesions. Since each NAIT location can have multiple herds present and a herd can be spread across multiple NAIT locations, a consistent herd identifier across the dataset could not be determined and herd-level clustering was ignored, only meat processing plant-level clustering was accounted for.

The results of the Multivariable model are presented in Table 2-5. Herd status, disease control area, year and season, and number of lifetime movements were statistically significantly associated with the submission of bTB-like lesions at routine carcass inspection.

Table 2-1 Frequencies and percentages, with 95% confidence intervals of cattle slaughtered (n=7,646,655), and bovine tuberculosis-like lesions reported at routine slaughter inspection (n=1199) between 1/1/2019 and 30/9/2021 within each of the categories of variables considered for analysis.

Variables	Category	Number of cattle slaughtered (n=7,646,655)	% of population slaughtered (95% CI <sup>a</sup> )	Number of bTB <sup>b</sup> -like lesions submitted (n=1199)	% of bTB-like lesions submitted (95% CI)
Sex	Male	662212	8.7 (8.6 - 8.7)	577	48.1 (45.3 - 51.0)
	Female	544505	7.1 (7.1 - 7.1)	610	50.9 (48.0 - 53.7)
	Unknown	6439938	84.2 (84.2 - 84.2)	12	1.0 (0.5 - 1.8)
Production Type	Dairy	3643359	47.6 (47.6 - 47.7)	513	42.8 (40.0 - 45.6)
	Beef	3872241	50.6 (50.6 - 50.7)	643	53.6 (50.7 - 56.5)
	Unknown	131055	1.7 (1.7 - 1.7)	43	3.6 (2.6 - 4.8)
Age	≥3	2189748	28.6 (28.6 - 28.7)	302	25.2 (22.7 - 27.7)
	<3	2713482	35.5 (35.4 - 35.5)	485	40.5 (37.7 - 43.3)
	Unknown	2743425	35.9 (35.8 - 35.9)	412	34.4 (31.7 - 37.1)
Season, Year	Summer 2019	723440	9.5 (9.4 - 9.5)	98	8.1 (6.7 - 9.9)
	Autumn 2019	967974	12.7 (12.6 - 12.7)	138	11.5 (9.8 - 13.5)
	Winter 2019	518902	6.8 (6.8 - 6.8)	94	7.8 (6.4 - 9.5)
	Spring 2019	483807	6.3 (6.3 - 6.3)	118	9.8 (8.2 - 11.7)
	Summer 2020	784628	10.3 (10.2 - 10.3)	141	11.7 (10.0 - 13.7)
	Autumn 2020	870129	11.4 (11.4 - 11.4)	141	11.7 (10.0 - 13.7)
	Winter 2020	561769	7.3 (7.3 - 7.4)	87	7.2 (5.9 - 8.9)
	Spring 2020	523977	6.9 (6.8 - 6.9)	97	8.1 (6.6 - 9.8)
	Summer 2021	519249	6.8 (6.8 - 6.8)	71	5.9 (4.7 - 7.4)
	Autumn 2021	963869	12.6 (12.6 - 12.6)	108	9.0 (7.5 - 10.8)
	Winter 2021	591589	7.7 (7.7 - 7.8)	84	7.0 (5.6 - 8.6)
	Spring 2021	137322	1.8 (1.8 - 1.8)	22	1.8 (1.2 - 2.8)
Lifetime Movements	≤2	3949085	51.6 (51.6 - 51.7)	665	55.5 (52.6 - 58.3)
	>2	3575306	46.8 (46.7 - 46.8)	492	41.0 (38.2 - 43.9)
	unknown	122264	1.6 (1.6 - 1.6)	42	3.5 (2.6 - 47.5)
Herd Status	Clear	6118586	80 (80.0 - 80.0)	1131	94.3 (92.8 - 95.5)
	Infected	15504	0.2 (0.2 - 0.2)	42	3.5 (2.6 - 4.7)
	Suspended	54141	0.7 (0.7 - 0.7)	26	2.2 (1.4 - 3.2)
	Unknown	1458424	19.1 (19.0 - 19.0)	0	0.0 (0.0 - 0.4)
Area Risk	Surveillance	5529462	72.3 (72.3 - 72.3)	779	65.0 (62.2 - 67.7)
	Movement control area	287847	3.8 (3.8 - 3.8)	103	8.6 (7.1 - 10.4)
	Special testing area annual	804091	10.5 (10.5 - 10.5)	198	16.5 (14.5 - 18.8)
	Special testing area biennial	999705	13.1 (13.0 - 13.1)	119	9.9 (8.3 - 11.8)
	Unknown	25550	0.3 (0.3 - 0.3)	0	0.0 (0.0 - 0.4)

<sup>a</sup> Confidence interval; <sup>b</sup> Bovine tuberculosis.

Table 2-2 Number of cattle slaughtered per bovine tuberculosis-like lesion submitted with 95% confidence intervals from 44 meat processing plants that submitted 1199 bovine tuberculosis-like lesions from 7,646,655 cattle at routine carcass inspection between 1/1/2019 and 30/9/2021. There was a statistically significant difference between bovine tuberculosis-like lesion submission rates by meat processing plant using generalised linear modelling with a likelihood ratio test p-value of <0.001.

Meat Processing Plant ID <sup>a</sup>	Number of cattle slaughtered per bTB <sup>b</sup> -like lesion submitted (95%CI <sup>c</sup> )
6216	8764 (6570 - 11723)
6494	253596 (39063 - 5000000)
6906	44726 (22676 - 91743)
7891	19253 (9756 - 39526)
7892	22427 (14245 - 35714)
7893	13155 (35714 - 21882)
8452	4206 (2208 - 8278)
9234	0 (0 - 173)
14497	9780 (4261 - 24038)
14994	117458 (18083 - 2500000)
15025	5692 (3826 - 8532)
15040	59631 (21692 - 185185)
15336	11720 (7524 - 18450)
15894	90368 (32895 - 285714)
16366	422 (393 - 453)
16924	6538 (4946 - 8666)
18196	8704 (6274 - 12136)
18234	13862 (5590 - 37594)
18239	196022 (48544 - 1111111)
18434	0 (0 - 80000)
18437	27940 (14663 - 54945)
23489	16471 (10684 - 25641)
25537	0 (0 - 14903)
30017	16428 (2530 - 312500)
30196	91588 (33333 - 285714)
30214	9902 (6798 - 14514)
31126	15336 (2362 - 294118)
31148	17006 (9200 - 32362)
32804	32754 (19380 - 56497)
36495	41906 (13158 - 161290)
36580	29003 (13423 - 66225)
43825	0 (0 - 86)
54880	4676 (3438 - 6382)
56368	0 (0 - 114)
56372	0 (0 - 23)
58783	0 (0 - 16)
59579	0 (0 - 111)
60768	0 (0 - 39)
64125	19170 (2952 - 370370)
75237	0 (0 - 3992)

111451	9650 (1486 - 185185)
117696	0 (0 - 116)
124656	0 (0 - 19)
129366	0 (0 - 429)

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<sup>a</sup> Identification; <sup>b</sup> Bovine tuberculosis-like; <sup>c</sup> Confidence interval

Table 2-3 Percentage of agreement between the disease control area of 1. the two last known locations before slaughter, and 2. the last known location and the birth location (and 95% confidence intervals), for the general population (n=7,646,655), for animals that were at the sending to slaughter location for less than 30 days (n=501,126), and for animals that were sent to slaughter from a saleyard (n=20,359)

	% Match (95% CI) <sup>a</sup>	% No match (95% CI)	% Unknown (95% CI)	Total cattle
<b>General population</b>				7646655
Disease control areas of the last two known locations before slaughter	29.7 (29.6 - 29.7)	12.8 (12.8 - 12.8)	57.5 (57.5 - 57.6)	
Disease control areas of the birth location and last known location before slaughter	27.9 (29.7 - 28.0)	11.0 (11.0 - 11.1)	61.0 (61.0 - 61.1)	
<b>Animals less than 30 days at sending to slaughter location</b>				501126
Disease control areas of the last two known locations before slaughter	41.9 (41.8 - 42.1)	10.2 (10.1 - 10.3)	47.9 (47.7 - 48.0)	
Disease control areas of the birth location and the last known location before slaughter	27.8 (27.7 - 27.9)	9.3 (9.2 - 9.3)	63.0 (62.8 - 63.1)	
<b>Animals sent to slaughter from Saleyards</b>				20359
Disease control areas of the last two known locations before slaughter	45.8 (45.1 - 46.5)	17.1 (16.6 - 17.6)	37.1 (36.4 - 37.8)	
Disease control areas of the birth location and the last known location before slaughter	25.0 (24.4 - 25.6)	13.7 (13.2-14.1)	61.4 (60.7 - 62.0)	

<sup>a</sup> Confidence interval

Table 2-4 Univariable logistic regression model outputs for risk-factors of bovine tuberculosis-like lesions submitted from cattle at routine carcass inspection between 1/1/2019 and 30/09/2021.

Variable	Category	Univariable analysis			
		Coefficient	Standard error	p-value	Odds Ratio (95%CI <sup>a</sup> )
Sex	Male	-	-	<b>&lt;0.001</b>	1 (ref.)
	Female	0.2515	0.0580	<0.001	1.28 (1.15 - 1.44)
Production Type	Dairy	-	-	<b>0.005</b>	1 (ref.)
	Beef	0.1649	0.0592	0.007	1.18 (1.05 - 1.32)
Age	≥3	-	-	<b>&lt;0.001</b>	1 (ref.)
	<3	0.2593	0.0733	<0.001	1.29 (1.12 - 1.49)
Season, year	Autumn 2019	-	-	<b>&lt;0.001</b>	1 (ref.)
	Summer 2019	-0.0511	0.1320	0.698	0.95 (0.72 - 1.23)
	Winter 2019	0.2395	0.1337	0.073	1.27 (0.98 - 1.65)
	Spring 2019	0.5370	0.1253	<0.001	1.71 (1.34 - 2.19)
	Summer 2020	0.2315	0.1197	0.053	1.26 (0.99 - 1.59)
	Autumn 2020	0.1280	0.1197	0.284	1.14 (0.90 - 1.44)
	Winter 2020	0.0827	0.1369	0.545	1.08 (0.83 - 1.42)
	Spring 2020	0.2612	0.1325	0.048	1.30 (1.0 - 1.68)
	Summer 2021	-0.0417	0.1460	0.774	0.96 (0.72 - 1.28)
	Autumn 2021	-0.2409	0.1284	0.060	0.79 (0.61 - 1.01)
	Winter 2021	-0.0040	0.1383	0.976	0.99 (0.76 - 1.31)
	Spring 2021	0.1166	0.2295	0.611	1.12 (0.72 - 1.76)
	Movements	≤2	-	-	<b>&lt;0.001</b>
>2		-0.2019	0.0594	<0.001	0.82 (0.73 - 0.92)
Herd Status	Clear	-	-	<b>&lt;0.001</b>	1 (ref.)
	Suspended	0.9550	0.1984	<0.001	2.60 (1.76 - 3.83)
	Infected	2.6873	0.1573	<0.001	14.79 (10.79 - 20)
Disease control area	Surveillance	-	-	<b>&lt;0.001</b>	1 (ref.)
	Movement control area	0.9323	0.1048	<0.001	2.54 (2.07 - 3.12)
	Special testing area annual	0.5584	0.0796	<0.001	1.74 (1.49 - 2.04)
	Special testing area biennial	-0.1685	0.0984	0.0935	0.84 (0.69 - 1.02)

<sup>a</sup> Confidence interval; Right aligned bold P values are the likelihood ratio significance test result for the variable; Left aligned P values are Wald statistics

Table 2-5 Multivariable logistic regression model outputs for risk-factors for submission of bovine tuberculosis-like lesions from cattle at routine carcass inspection between 1/1/2019 and 30/09/2021

Variable	Category	Coefficient	S.E <sup>a</sup>	p-value	Odds ratio (95%CI <sup>b</sup> )
Disease control area	Surveillance	-	-	-	1 - (ref.)
	Movement Control Area	-0.32	0.13	0.018	0.73 (0.56 - 0.95)
	Special Testing Area - Annual	-0.01	0.08	0.874	0.99 (0.84 - 1.16)
	Special Testing Area - Biennial	-0.15	0.10	0.141	0.86 (0.71 - 1.05)
Herd Status	Clear	-	-	-	1 - (ref.)
	Infected	1.36	0.20	<0.001	3.9 (2.66 - 5.73)
	Suspended	0.73	0.20	<0.001	2.08 (1.4 - 3.1)
Season, Year	Autumn 2019	-	-	-	1 - (ref.)
	Summer 2019	0.08	0.13	0.567	1.08 (0.83 - 1.41)
	Winter 2019	0.31	0.14	0.024	1.36 (1.04 - 1.78)
	Spring 2019	0.29	0.13	0.022	1.34 (1.04 - 1.73)
	Summer 2020	0.32	0.12	0.009	1.38 (1.08 - 1.75)
	Autumn 2020	0.28	0.12	0.022	1.32 (1.04 - 1.68)
	Winter 2020	0.03	0.14	0.817	1.03 (0.79 - 1.36)
	Spring 2020	-0.04	0.14	0.795	0.97 (0.74 - 1.26)
	Summer 2021	0.09	0.15	0.559	1.09 (0.81 - 1.46)
	Autumn 2021	-0.20	0.13	0.130	0.82 (0.64 - 1.06)
	Winter 2021	0.07	0.14	0.598	1.08 (0.82 - 1.42)
	Spring 2021	-0.40	0.24	0.085	0.67 (0.42 - 1.06)
Number of lifetime movements	≤2	-	-	-	1 - (ref.)
	>2	-0.12	0.06	0.042	0.88 (0.79 - 1)
Variance (Std.dev <sup>c</sup> )	Meat Processor				1.84 (1.35)

<sup>a</sup> Standard error; <sup>b</sup> Confidence interval; <sup>c</sup> Standard deviation

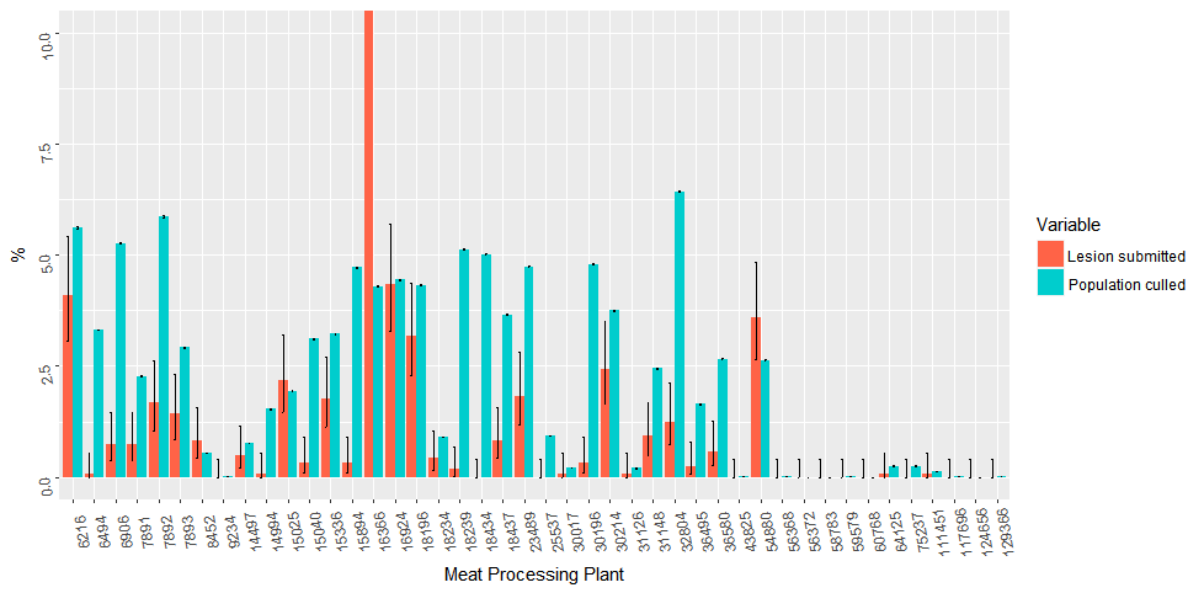


Figure 2-2 Percentage of population slaughtered by meat processing plant (n=7,646,655), and percentage by plant of bovine tuberculosis-like lesions submitted (n=1199) between 1/1/2019 and 30/9/2021. Meat processing plant 16366 submitted 64.8% (95%CI 62.0% – 67.5%) of bovine tuberculosis like lesions, the y-axis is limited at 10%.

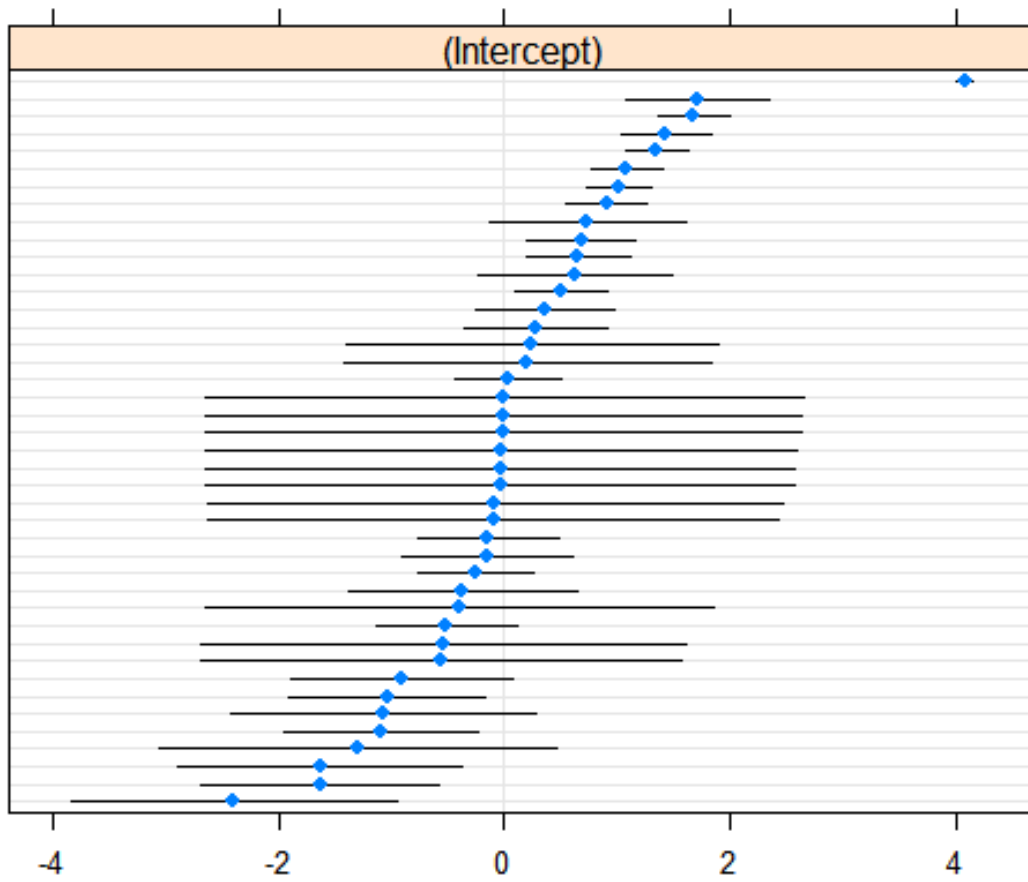


Figure 2-3 Random intercept value (with 95% confidence intervals) for the random effect of meat processing plant in the multivariate model describing the risk of bovine tuberculosis-like lesion submission from cattle at routine carcass inspection between 1/1/2019 and 30/09/2021.

## 2.4 Discussion

In this study, potential risk-factors for bTB-like lesions submitted from cattle at routine carcass inspection between 1 January 2019 and 31 October 2021 were evaluated. The overall submission rate of bTB-like lesions was one submission for every 6378 carcasses inspected. Cattle from an infected or suspended status herd or with more than two lifetime movements had the highest risk of bTB-like lesions being submitted by meat inspectors at routine carcass inspection. Animals sent to slaughter from within a movement control area were less likely to have a bTB-like lesion submitted than cattle from a surveillance testing area. The odds of submitting bTB-like lesions, were higher in the period from winter 2019 through to autumn 2020, compared to autumn 2019. The baseline odds of bTB-like lesion submission differed between meat processing plants, indicating that factors not accounted for in the model and that may be clustered by or specific to meat processing plants contributed to the observed outcome.

The overall bTB-like submission rate from cattle at routine carcass inspection in this study was 6.3% of that achieved in Ireland (1 bTB-like lesion per 400 cattle slaughtered) (Olea-Popelka et al., 2012) where bTB is more prevalent than in New Zealand, and 13.6% of that achieved in Australia (1 per 866 cattle slaughtered) during the later stages of bTB eradication where bTB was less prevalent than in New Zealand (More et al., 2015). The higher bTB-like lesion submission rate in Ireland and Australia, despite the differences in bTB prevalence, suggests there are factors other than true bTB prevalence that influences the detection and submission of bTB-like lesions. There was also a higher variability in crude bTB-like lesion submission rates between meat processing plants in New Zealand (from 1 per 422 cattle slaughtered to 1 per 253,596 cattle slaughtered) compared to those reported in other countries where bTB is endemic. Crude submission rates by meat processing plants are reported at 1 per 185 - 1250 cattle slaughtered in Northern Ireland (Pascual-Linaza et al., 2017), 1 per 736 - 6400 in Great Britain (Shittu et al., 2013), and 1 per 620 – 50000 in Castilla y Leon, Spain (Pozo et al., 2021). This study did not adjust bTB-like lesion submission rates based on the population slaughtered so there may be a higher degree of heterogeneity between meat processing plants slaughtering cattle in New Zealand compared to other countries explaining the greater difference in crude bTB-like lesion submission rates. Adjusting the crude bTB-like lesion submission rates between meat processing plants based on population of cattle slaughtered may enable a more direct comparison between meat processing plants in New Zealand.

Animals sent to slaughter from a movement control area were less likely to have bTB-like lesions submitted from routine carcass inspection than animals sent to slaughter from surveillance testing areas. This finding contrasts with those of other countries where cattle sent to slaughter from areas with a higher incidence of bTB are more likely to have bTB-like lesions disclosed (Frankena et al., 2007; Pascual-Linaza et al., 2017; Shittu et al., 2013). There may be confounding factors that were not included in the analysis (such as age) that explains this apparent protective effect. There may be geographical differences in risk of bTB-like lesion development that are not related to the risk of acquiring bTB from wildlife which does carry an area risk. The prevalence of bTB in countries where area risk is associated with an increased risk of bTB-like lesion submissions is higher than in New Zealand, which may explain why there is an increased risk in these countries not observed in New Zealand.

The current study included the number of lifetime movements as a proxy for contact with other livestock. Animals with three or more lifetime movements had a lower risk of bTB-like lesion submission than those with less than three movements. The reduction in risk with increased movements is in contrast to Northern Ireland, where purchased was used as a proxy for increased opportunity to become infected with bTB and was associated with a higher risk of bTB-like lesion submission (Pascual-Linaza et al., 2017). Age is one potential confounder that might contribute to this apparent protective effect of lifetime movements in New Zealand. Age was excluded from the multivariate analysis due to missing data. However, in the univariate analysis, younger animals had a higher risk of bTB-like lesion submission. Younger animals may have fewer lifetime movements than older animals. Younger animals were at higher risk for bTB-like lesion submissions than older animals in Northern Ireland (Pascual-Linaza et al., 2017) which supports this theory, but older animals (2+) were more likely to have lesions reported at routine carcass inspection in Spain (Pozo et al., 2021). The prevalence and age predisposition of bTB-like lesion differentials may influence the effect of age on bTB-like lesion submission between countries. Considering the low prevalence of bTB in New Zealand, a higher number of lifetime movements may be a proxy for age rather than the opportunity to become infected with bTB from contact with infected cattle or wildlife.

The current study found that cattle from infected or suspended status herds had a higher risk of bTB-like lesion submissions from cattle at routine slaughter than those from clear herds. Similarly, in other countries, the odds of submission were higher among animals from herds with a recent history of infection with bTB (Frankena et al., 2007; Pascual-Linaza et al., 2017). Higher levels of on-farm testing in infected or suspended herds may favour the detection and removal of bTB infected animals which may decrease the risk of true bTB lesions being reported at slaughter which doesn't support this finding. There is no biological plausibility for infected or suspended status herds having

higher risk of bTB-like lesion submission considering that with such a low prevalence of disease, most bTB-like lesions are non-tuberculous. It is possible meat inspectors are aware they are examining carcasses from infected or suspended status herds and be more likely to submit a bTB-like lesion for confirmatory testing.

During the current study period, an increased risk for bTB-like lesion submissions from cattle at routine carcass inspection was observed beginning autumn 2019 and ending winter 2020. Unlike other countries where season was a risk for bTB-like lesion submission (Pascual-Linaza et al., 2017), in New Zealand there was a year period beginning winter 2019 when a higher risk of bTB-like lesion submissions was observed. During June (winter) 2019, a “refresher training” programme was carried out at meat processing plants across New Zealand, educating meat inspectors on the role they play in bTB surveillance and recognition of bTB-like lesions. Difficulty in maintaining motivation of meat inspectors to submit bTB-like lesions for confirmation when the prevalence of disease is low had been suggested as a reason for decline in bTB-like lesion submissions in Australia (Sergeant et al., 2017), and may be contributing to low bTB-like lesion submission rates in New Zealand.

Communication and training may have contributed to the immediate increase in bTB-like lesion submissions around that time. Factors like attendance at training courses have been associated with higher bTB-like lesion detection rates in Spain (Garcia-Saenz et al., 2015). A lack of co-ordination between authorities responsible for food safety and animal health has been identified as limiting slaughter surveillance for bTB in Spain (Napp et al., 2019). Providing ongoing training and having regional liaison officers dedicated to communicating with meat inspection teams and public health officials contributed to the success of the National Granuloma Submission Programme in Australia (More et al., 2015). An increase in bTB-like lesion submission rates following training and communication in New Zealand could suggest this is an effective method for enhancing bTB surveillance. However, the apparent short-lived nature of the response may indicate the need for more regular or meaningful engagement with those participating in slaughter surveillance for bTB.

The result of the multivariate model shows that after adjusting for covariates influencing the risk of bTB detection, there is still a difference in baseline risk of bTB-like lesions submitted at routine carcass inspection between meat processing plants. The difference in baseline risk could be a result of factors not considered in the model and specific to meat processing plants, as seen in Ireland (Frankena et al., 2007; Olea-Popelka et al., 2012), Northern Ireland (Pascual-Linaza et al., 2017), Great Britain (Shittu et al., 2013), United States of America (Kaneene et al., 2006), Catalonia Spain (Garcia-Saenz et al., 2015) and Castilla y Leon Spain (Pozo et al., 2021). Meat processing plant-specific factors influencing bTB-like lesion submission rates have not been quantified. However, attendance at training courses, number of meat inspectors, speed of the slaughter chain, inspection

facilities and experience of the meat inspectors have all been suggested as factors that might influence the difference in bTB-like lesion submission rates between meat processing plants that cannot be explained by animal risk-factors (Garcia-Saenz et al., 2015; Male Here et al., 2022; Olea-Popelka et al., 2012; Pascual-Linaza et al., 2017; Pozo et al., 2021; Shittu et al., 2013). Differences in baseline risk of bTB-like lesion submission may mean that a proportion of truly infected bTB cattle go undetected, which could jeopardise national efforts to control bTB. Identification and quantifying meat processing plant-specific factors that influence the risk of bTB-like lesion submission rates may be useful to improve or assist with standardizing bTB-like lesion submissions across meat processing plants to enhance slaughter surveillance for bTB in New Zealand. Ethical challenges such as discriminating between industry, regulatory body and private business risk-factors may be prohibitive and could explain the lack of research investigating these risk-factors. Developing a policy or a programme that circumvents the need to quantify the meat processing plant-specific risks for bTB-like lesion submissions may be a way to address this constraint. The National Granuloma Submission Programme developed in Australia during the later stages of eradication attempted to standardise bTB-like lesion submission rates across the country without targeting plant-specific factors specifically (Sergeant et al., 2017). A similar programme could be considered to address this challenge in New Zealand.

## 2.5 Conclusion

There are meat processor-specific factors that influence whether a bTB-like lesion is submitted. In addition to meat processing plant factors, a bTB-like lesion was more likely to be submitted from an animal that came from an infected or suspended status herd, moved less than three times prior to slaughter, and came from an area outside of movement control areas. The overall low submission rate found in our study may indicate sub-optimum sensitivity of routine carcass inspection to identify potential bTB lesions in NZ. Identifying and targeting plant-specific factors that are limiting bTB-like lesion submissions could be used to enhance slaughter surveillance for bTB in New Zealand. Ongoing training and engagement with those involved in slaughter surveillance for bTB is recommended to maintain elevated bTB-like lesion submission rates and support eradication of bTB in New Zealand.

- 3 Risk factors for confirmation of *Mycobacterium bovis* from bovine tuberculosis-like lesions submitted from routine carcass inspection in cattle in New Zealand

### 3.1 Introduction

In New Zealand, the bTB surveillance system consists of on-farm livestock testing and slaughter surveillance for the detection of bTB (Biosecurity (National Bovine Tuberculosis Pest Management Plan) Order 1998). Under New Zealand legislation, during routine carcass inspection, meat inspectors are required to palpate and incise lymph nodes where bTB is commonly identified. bTB is notifiable by law and the requirement to report suspect bTB forms the basis of the slaughter surveillance component of the bTB surveillance system. Confirmation of *Mycobacterium bovis* from bTB-like lesions detected at routine carcass inspection facilitates the control of bTB in New Zealand. Between 1 January 2019 and 30 September 2021 there were 1199 bTB-like lesions submitted to the bTB management agency from cattle that were subjected to routine carcass inspection as described in Chapter 2. When bTB is confirmed, rigorous on-farm testing is carried out to detect and remove further infection within the herd of origin. On-farm testing of herds where bTB infection could have originated or spread to is also carried out to detect and prevent further spread of bTB.

Routine carcass inspection in New Zealand is the primary mechanism for detecting bTB in some class of stock such as prime beef animals (OSPRI, 2021b). Cattle which are slaughtered in New Zealand are therefore not subjected to a uniform intensity of ante-mortem screening. In 1998, area livestock testing policies were implemented to account for the difference in area risk of acquiring bTB infection from local wildlife. Herds across New Zealand are tested at different frequencies because of this area risk which is in contrast to other countries, Northern Ireland for example, where bTB is endemic and are tested annually (Pascual-Linaza et al., 2017). Slaughter surveillance is the primary mechanism for detecting bTB in countries that have eradicated bTB and a transition from on-farm testing to predominantly slaughter surveillance during the later stages of eradication is a logical progression towards this change (El Allaki et al., 2016; Napp et al., 2019; Ramanujam et al., 2021; Sergeant et al., 2017). New Zealand's bTB surveillance system has begun a transition from on-farm testing to predominately slaughter surveillance as progress is made towards achieving bTB eradication. New Zealand's bTB eradication milestones are: bTB freedom from livestock by 2026, bTB freedom from the wildlife vector by 2040, and total biological eradication by 2055 (Biosecurity (National Bovine Tuberculosis Pest Management Plan) Order 1998). Hence, there is an increasing emphasis on the importance of slaughter surveillance for bTB in New Zealand.

The aim of this study is to assess risk-factors associated with confirmation of *Mycobacterium bovis* (bTB-positive) from bTB-like lesions submitted from routine carcass inspection. Identifying risk-factors for bTB-like lesions confirmed as bTB positive may identify possible enhancements to on-

farm surveillance strategies or provide evidence for implementing an enhanced, risk-based approach to slaughter surveillance.

## 3.2 Materials and methods

The dataset used in this chapter is a subset of data used in the analysis for chapter 2 and will be described briefly in this chapter. Forty-four meat processing plants processed cattle between 1 January 2019 and 30 September 2021 and met the inclusion criteria (see 2.2). The dataset used in this analysis came from bTB-like lesions reported during routine carcass inspection from 31 meat processing plants. When a bTB-like lesion was reported during routine carcass inspection, the lesion was sampled, and fresh and fixed specimens were prepared. The samples were sent to one commercial veterinary laboratory for histopathological examination. If the lesion was histologically “Suspicious of bTB” or “Typical of bTB”, the fresh sample was referred to a different laboratory for PCR and culture to confirm the presence of bTB. Animal details from routine carcass inspection and laboratory results were entered into the Disease Management System (DMS) database. The outcome variable was a binary variable coded as 1 for “bTB confirmed” or 0 for “Not TB”. A bTB-like lesion was confirmed as bTB if positive to PCR or culture or both. A bTB-like lesion was “Not TB” if the lesion was histologically “Not tuberculosis” or negative to PCR and culture. Herd-level data and accompanying individual animal bTB data was extracted from the DMS database.

Individual animal data was extracted from the National Animal Identification and Traceability (NAIT) database for cattle with bTB-like lesions submitted during the study period. Individual animal risk-factors included in the statistical analysis were sex, age, number of lifetime movements between farms, the bTB herd status of the herd where the animal was sent to slaughter from, the disease control area the animal was sent to slaughter from, the season of slaughter, meat processing plant and herd. A herd may supply cattle to more than one meat processing plant for slaughter, therefore a herd may have animals with bTB-like lesions submitted from several meat processing plants.

The data sets were combined using the lifetime animal identification number and collated using R Statistical software (v4.1.2; R Core Team 2021).

### 3.2.1 Description of explanatory variables

#### 3.2.1.1 *Disease control area*

In New Zealand, bTB management varies according to the risk of acquiring bTB from the wildlife vector. These areas are referred to as disease control areas and are presented in Figure 2-1. There are four disease control areas which are described in detail in Chapter 2 (see section 2.2.1.1 ). Briefly, in order of highest risk to lowest risk the area risk categories are: 1. Movement control area, 2. Special testing area annual, 3. Special testing area biennial, 4. Surveillance testing area. Special

testing area annual and special testing biennial were collapsed into one category as there were no bTB-positive cases from the special testing area annual. The levels used in analysis were surveillance, movement control area, and special testing area annual or biennial.

For all cattle in the study population, the disease control area of the last location an animal was at before slaughter was used. For those sent to slaughter from a saleyard, the disease control area of the location they were at before the saleyard was used. A description of the distribution of time at the last location before slaughter, and the relationship between the disease control area of last location, location before last location and birth location was provided Table 2-3.

#### *3.2.1.2 Age*

Age at slaughter was determined by the difference in days between date of birth and date of slaughter. Date of registration was used as a proxy for date of birth where date of birth was unknown. Age was categorized into <3 years and ≥3 years. The high proportion of missing age precluded the use of age in the multivariable model, but we still describe age in the descriptive results.

#### *3.2.1.3 Production type*

Production type is a compulsory field in the NAIT database and is entered as Dairy or Beef. Slaughter levies for the bTB programme and the NAIT programme are collected based on production type. Production type is declared upon registration in the NAIT system but can be changed during the lifetime of the animal.

#### *3.2.1.4 Herd status*

The Herd status has been described in detail in Chapter 2 (See Section 2.2.1.5). Herd status of the sending to slaughter herd was known for all the observations within this dataset, unlike the dataset of Chapter 2 where an assumption of herd status was made. Breeding herds and meat production herds free from bTB infection were collapsed into one category as there was no statistically significant difference between the number of bTB confirmed cases between breeding and meat production levels. The levels of herd status for this analysis were clear, suspended, or infected.

#### *3.2.1.5 Sex*

Sex is a voluntary field in the NAIT database. When an animal with a bTB-like lesion was reported at routine carcass inspection, necropsy and animal details were entered into DMS database. The levels were female or male.

#### *3.2.1.6 Number of lifetime movements*

Farmers have a legal obligation to record all animal movements in the NAIT database. Lifetime animal movements was considered a proxy for contact with other livestock. Number of movements were categorized into ≤2 movements or >2 movements.

### 3.2.1.7 Temporal variable

Season and year were both considered for inclusion. The dataset did not cover three calendar years and to avoid drawing conclusions based on a comparison of incomplete seasons and years, a variable that captured both the year and season was created. Summer months included December, January, and February. Autumn months included March, April, and May. Winter months included June, July, and August. Spring months included September, October, and November.

### 3.2.2 Data analysis

Data analysis was performed using R (v4.1.2; R Core Team 2021). Continuous data were assessed for normality using histograms. Non-normally distributed continuous variables were converted to a categorical variable using the quartiles. For categorical variables, the Pearson's Chi-squared test of proportions was used to compare the risk of bTB-like lesion confirmation between levels. The Fisher's exact test was used where cell counts in the two-by-two tables were less than 5. Logistic regression with bTB confirmed as the outcome variable was used to assess the relationship between explanatory variable categories when the Fisher's Exact test was too computationally challenging. Categorical variable levels were collapsed if there were no statistically significant differences between them using a p-value of  $<0.05$ , and if it was biologically appropriate.

A bivariate logistic regression model was carried out for all explanatory variables, using the submission of a suspect bTB lesion following routine carcass inspection (0 or 1) as the outcome. Results were presented as odds ratios and its 95% confidence interval. All explanatory variables with a likelihood ratio chi-square test p-value of  $<0.2$  were included in the multivariable model building.

Multivariable analyses were performed using a manual backwards elimination procedure to obtain a parsimonious model with meat processing plant fitted as a random effect at the beginning of the model building. The explanatory variable with the least significant p-value was removed one at a time until only variables with a p-value of  $< 0.05$  remained. A second multivariate model was generated nesting herd within meat processor as a random effect for comparison. All explanatory variables with a likelihood ratio chi-square test p-value of  $<0.2$  were included in the multivariate model with herd nested within meat processor as a random effect. The explanatory variable with the least significant p-value was removed one at a time until only variables with a p-value of  $< 0.05$  remained.

Collinearity between explanatory variables was assessed visually during data exploration for variables where collinearity may exist, but no obvious issue was detected. Collinearity between explanatory variables included in the final models was further evaluated using the variance inflation

factor. All explanatory variable variance inflation values were less than five so collinearity was not considered an issue.

### 3.3 Results

There were 1199 bTB-like lesions submitted for confirmation between 1 January 2019 and 30 September 2021. Of the 1199 bTB-like lesions submitted for confirmation, 20 (1.7%) were confirmed as bTB positive. bTB positive samples came from 12 herds; 11 herds had one bTB-like lesion confirmed as bTB positive, and one herd had nine bTB-like lesions confirmed as bTB positive. There were ten herds that were newly identified as containing bTB infection during the study period. There were 758 herds that had between one and 29 bTB-like lesions submitted during the study period. Twenty one percent of herds (n=163) that had bTB-like lesions submitted during the study period had more than one lesion submitted. There were 20 herds (2.6%) that had bTB-like lesions submitted from two separate meat processing plants. All explanatory variables of statistical significance in the final multivariable models were known for ninety-nine percent of animals (1187 of 1199). Unknown variables are included in the table of descriptive statistics Table 3-1.

There was a statistically significant difference in bTB confirmation rates between meat processing plants from 0.0% (95%CI 0.0 – 94.5%) to 50.0% (95%CI 9.5 – 90.5%). Confirmation rates by meat processing plant are presented in Figure 3-1. A description of the numbers and percentages of lesions submitted within each category of the explanatory variables included in the analysis are presented in Table 3-1. Univariable analysis of each explanatory variable was carried out and the results are presented in Table 3-2. Production type was the only explanatory variable with a p-value of >0.2 and was not included in the multivariable model building.

Meat processing plant was fitted in the multivariate model as a random effect to account for clustering within meat processing plants. The random intercept of meat processing plant as a random effect (Figure 3-2) displays the baseline risk of confirmation of bTB from bTB-like lesions submitted at routine carcass inspection considering all other covariates. This represents the variation per plant of the baseline odds of confirmation of bTB from bTB-like lesions submitted at routine carcass inspection, at fixed levels of all other model covariates.

Two multivariable models were built for comparison. Sex, herd status and area risk were explanatory variables statistically significantly associated with the risk of bTB confirmation in the model with meat processor as a random effect. Sex was the only explanatory variable considered statistically significantly associated with the risk of bTB confirmation in the model with herd nested within meat

processor as a random effect. The results of the two multivariable analyses are presented in Table 3-3.

Table 3-1 Frequencies and percentages, with 95% confidence intervals of bovine tuberculosis like-lesions submitted from cattle at routine carcass inspection (n=1199) and confirmed as bovine tuberculosis positive (n=20) between 1/1/2019 and 30/09/2021 within the categories of variables considered for analysis.

Variables	Category	Number of Lesions submitted (n=1199)	Percentage and 95% CI <sup>a</sup> of lesions submitted (n=1199)	Number confirmed as bTB <sup>b</sup> (n=20)	Percentage and 95% CI of lesions Confirmed bTB (n=20)
Sex	Male	577	48.1 (45.3 - 51.0)	5	25.0 (9.6 - 49.4)
	Female	610	50.9 (48.0 - 53.7)	15	75.0 (50.6 - 90.4)
	Unknown	12	1.0 (0.5 - 1.8)	0	0 (0.0 - 20.0)
Production Type	Dairy	513	42.8 (40.0 - 45.6)	8	40.0 (19.9 - 63.6)
	Beef	643	53.6 (50.7 - 56.5)	11	55.0 (32.0 - 76.2)
	Unknown	43	3.6 (2.6 - 4.8)	1	5.0 (0.3 - 26.9)
Age	≥3	302	25.2 (22.7 - 27.7)	9	45.0 (23.8 - 67.9)
	<3	485	40.5 (37.7 - 43.3)	5	25.0 (9.6 - 49.4)
	Unknown	412	34.4 (31.7 - 37.1)	6	30.0 (12.8 - 54.3)
Season.Year	Summer 2019	98	8.1 (6.7 - 9.9)	3	15.0 (4.0 - 38.9)
	Autumn 2019	138	11.5 (9.8 - 13.5)	5	25.0 (9.6 - 49.4)
	Winter 2019	94	7.8 (6.4 - 9.5)	1	5.0 (0.3 - 26.9)
	Spring 2019	118	9.8 (8.2 - 11.7)	0	0 (0.0 - 20.0)
	Summer 2020	141	11.7 (10.0 - 13.7)	1	5.0 (0.3 - 26.9)
	Autumn 2020	141	11.7 (10.0 - 13.7)	5	25.0 (9.6 - 49.4)
	Winter 2020	87	7.2 (5.9 - 8.9)	0	0 (0.0 - 20.0)
	Spring 2020	97	8.1 (6.6 - 9.8)	1	5.0 (0.3 - 26.9)
	Summer 2021	71	5.9 (4.7 - 7.4)	1	5.0 (0.3 - 26.9)
	Autumn 2021	108	9.0 (7.5 - 10.8)	3	15 (4.0 - 38.9)
	Winter 2021	84	7.0 (5.6 - 8.6)	0	0 (0.0 - 20.0)
	Spring 2021	22	1.8 (1.2 - 2.8)	0	0 (0.0 - 20.0)
Herd Status	Clear	1131	94.3 (92.8 - 95.5)	9	45.0 (23.8 - 67.9)
	Infected	42	3.5 (2.6 - 4.7)	10	50.0 (29.9 - 70.1)
	Suspended	26	2.2 (1.4 - 3.2)	1	5.0 (0.3 - 26.9)
Lifetime Movements	≤2	665	55.5 (52.6 - 58.3)	15	75.0 (50.6 - 90.4)
	>2	492	41.0 (38.2 - 43.9)	4	20.0 (6.6 - 44.3)
	unknown	42	3.5 (2.6 - 47.5)	1	5.0 (0.3 - 26.9)
Disease Control Area	Surveillance	779	65.0 (62.2 - 67.7)	3	15.0 (4.0 - 38.9)
	Movement control area	103	8.6 (7.1 - 10.4)	15	75.0 (50.6 - 90.4)

Special testing area annual and biennial	317	26.4 (24.0 - 29.0)	2	10.0 (1.7 - 33.1)
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<sup>a</sup> Confidence interval; <sup>b</sup> Bovine tuberculosis

Table 3-2 Univariable (logistic regression) model outputs for risk-factors for confirmation of bovine tuberculosis from bovine tuberculosis-like lesions submitted from cattle at routine carcass inspection between 1/1/2019 and 30/09/2021.

Variable	Category	Univariable analysis				
		Coefficient	Standard error	p-value	Odds Ratio (95%CI <sup>a</sup> )	
Sex	Male	-	-	<b>0.029</b>	1 (ref.)	
	Female	1.05	0.5627	0.033	2.88 (1.04 - 7.99)	
Production Type	Dairy	-	-	<b>0.840</b>	1 (ref.)	
	Beef	0.09	0.46848	0.841	1.10 (0.44 - 2.75)	
Season.Year	Autumn 2019	-	-	<b>0.074</b>	1 (ref.)	
	Summer 2019	0.56	0.74	0.814	0.84 (0.19 - 3.60)	
	Winter 2019	-1.25	1.10	0.257	0.28 (0.032 - 2.49)	
	Spring 2019	-17.28	0.99	0.992	3.11e-08 (0.0 - Inf)	
	Summer 2020	-1.66	1.10	0.132	0.19 (0.212 - 1.65)	
	Autumn 2020	-0.02	0.64	0.972	0.97 (0.27 - 3.45)	
	Winter 2020	-17.28	1900.89	0.993	3.11e-08 (0.0 - Inf)	
	Spring 2020	-1.28	1.10	0.245	0.27 (0.03 - 2.41)	
	Summer 2021	-0.96	1.11	0.381	0.38 (0.04 - 3.31)	
	Autumn 2021	-0.27	0.74	0.711	0.76 (0.17 - 3.25)	
	Winter 2021	-17.28	1934.54	0.993	3.11e-08 (0.0 - Inf)	
	Spring 2021	-17.28	3780.12	0.996	3.11e-08 (0.0 - Inf)	
	Herd Status	Clear	-	-	<b>&lt;0.001</b>	1 (ref.)
		Infected	3.66	0.4932	<0.001	38.96 (14.82 - 102.42)
Suspended		1.60	1.07	0.134	4.99 (0.61 - 40.87)	
Lifetime Movements	≤2	-	-	<b>0.046</b>	1 (ref.)	
	>2	1.03	0.5659	0.0674	0.36 (0.12 - 1.08)	
Area Risk	Surveillance area	-	-	<b>&lt;0.001</b>	1 (ref.)	
	Movement control area	3.78	0.6424	<0.001	44.09 (12.52 - 155.29)	
	Special testing area annual and biennial	0.49	0.9153	0.588	1.64 (0.27 - 9.88)	

<sup>a</sup> Confidence interval; Right aligned bold P values are the likelihood ratio significance test result for the variable; Left aligned P values are Wald statistics

Table 3-3 Multivariable (logistic regression) model outputs for risk-factors for confirmation of bovine tuberculosis from bovine tuberculosis-like lesions submitted from cattle at routine carcass inspection between 1/1/2019 and 30/09/2021

Variable	Category	Random Effect = Meat processor				Random Effect = Meat Processor (herd)			
		Coefficient	S.E <sup>a</sup>	P value	Odds ratio (95%CI <sup>b</sup> )	β-coefficient	S.E	P value	Odds ratio (95%CI)
Sex	Male	-	-	-	1 (ref.)	-	-	-	1 (ref.)
	Female	1.48	0.65	0.023	4.39 (1.23 - 15.72)	3.08	1.15	0.007	21.75 (2.27 - 208.24)
Herd Status	Clear	-	-	-	1 (ref.)	-	-	-	-
	Infected	2.11	0.70	0.002	8.26 (2.09 - 32.59)	-	-	-	-
	Suspended	2.07	1.44	0.153	7.90 (0.46 - 135.15)	-	-	-	-
Disease control area	Surveillance area	-	-	-	1 (ref.)	-	-	-	-
	Movement control area	4.4	1.36	0.001	81.47 (5.5 - 1192.37)	-	-	-	-
	Special testing area annual and biennial	0.52	1.25	0.677	1.68 (0.14 - 19.47)	-	-	-	-
Variance (Std.dev <sup>c</sup> )	Meat processor	7.19 (2.68)				<0.001 (<0.001)			
	Herd	-				1.13 x 10 <sup>7</sup> (33.6)			

<sup>a</sup> Standard error; <sup>b</sup> Confidence interval; <sup>c</sup> Standard deviation

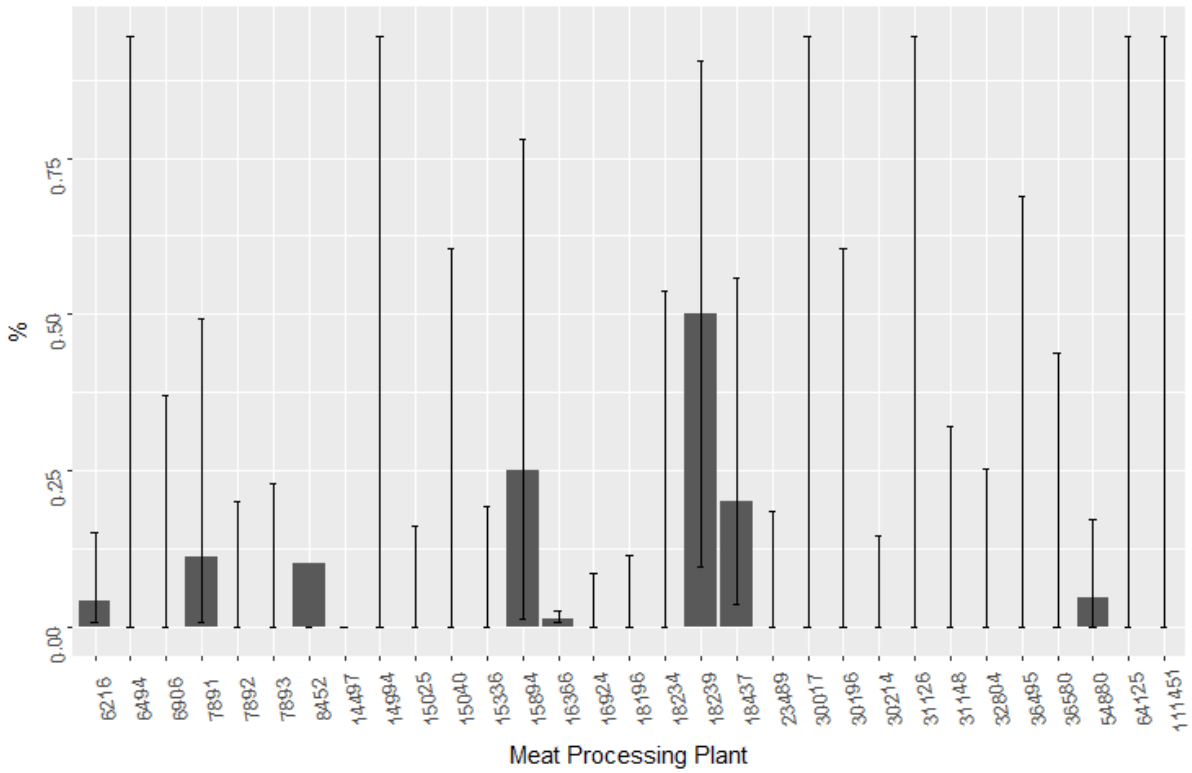


Figure 3-1 The confirmation rate and error bars indicating the 95% confidence intervals of 1199 bovine tuberculosis-like lesions submitted from cattle at routine carcass inspection between 1/1/2019 and 30/09/2021 by meat processing plant. There was a statistically significant difference between the confirmation rates of bovine tuberculosis-like lesions submitted by meat processing plant using the Fisher's exact test with a p-value <0.001.

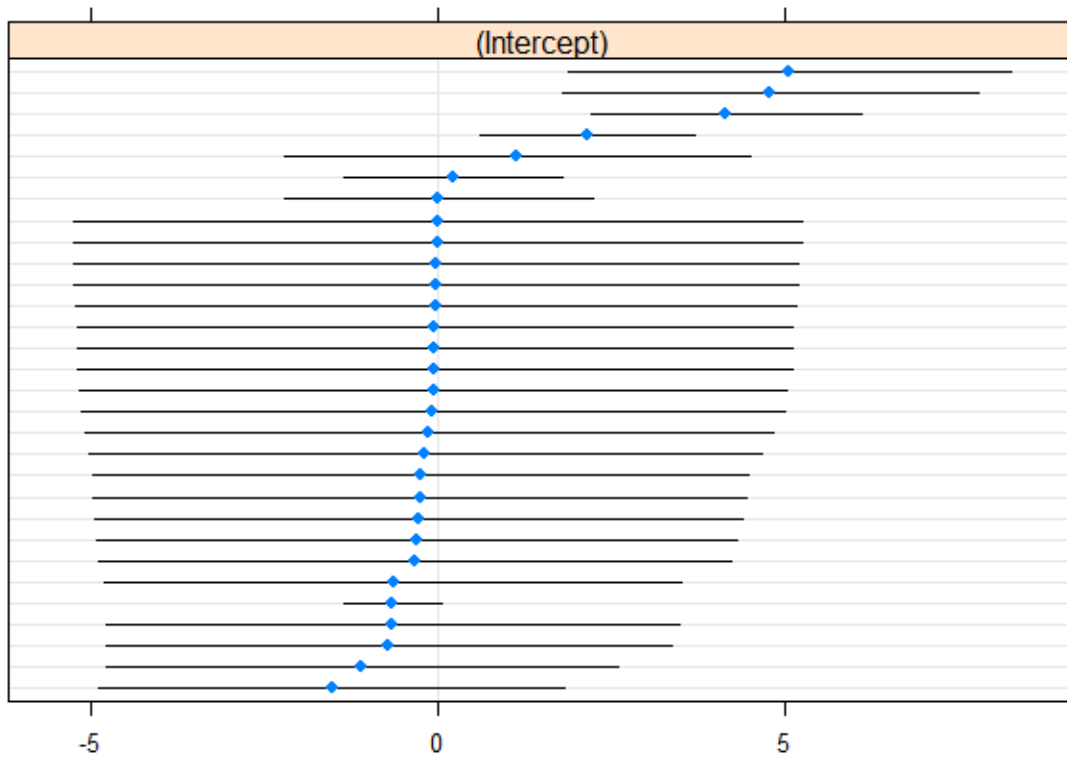


Figure 3-2 The random intercept value and error bars indicating the 95% confidence interval of Meat processing plant fitted as a random effect in the multivariate model describing the risk of bovine tuberculosis confirmation from 1199 bovine tuberculosis-like lesions submitted from cattle at routine carcass inspection between 1/1/2019 and 30/09/20

### 3.4 Discussion

In this study risk-factors for laboratory confirmation of bTB from bTB-like lesions submitted from cattle at routine carcass inspection between 1 January 2019 and 31 October 2021 were evaluated. bTB was confirmed in 1.7% of bTB-like lesions submitted at routine carcass inspection during the study period. Female cattle from infected status herds within movement control areas had the highest risk of confirmation of bTB from lesions submitted by meat inspectors. Confirmed bTB lesions were clustered within herds, nine of 20 confirmed bTB positive bTB-like lesions from routine carcass inspection were from a single infected herd. In the model with herd nested within meat processing plant, the herd random effect absorbs a lot of the residual variance that was attributed to meat processing plant in the model without herd nested as a random effect (Table 3-3). Removing herd as a nested random effect meant that risk-factors that are probably clustered by herd are identified in the model outputs and for this reason is the preferred model. Identifying herd-level risk-factors for bTB confirmation from bTB-like lesions reported at routine carcass inspection such as herd status and the disease control area they were sent to slaughter from may be used to enhance surveillance efforts.

The overall bTB confirmation rate from bTB-like lesions submitted at routine carcass inspection of 1.7% in this study is lower than reported from other countries where bTB is endemic; in Great Britain for example, 67% of bTB-like lesions reported at routine carcass inspection were confirmed as bTB positive (Shittu et al., 2013), in Portugal, 47.2% were confirmed as bTB positive (Gonçalves et al., 2022), while in Ireland and Northern Ireland, confirmation rates between 62% and 69% were reported (Frankena et al., 2007; Olea-Popelka et al., 2012; Pascual-Linaza et al., 2017). Countries with high bTB confirmation rates from bTB-like lesions reported at routine carcass inspection express concern that the apparently high specificity of routine carcass inspection for bTB in turn suggests a lower sensitivity. Confirmation rates are low in NZ, but that does not necessarily equate with a high sensitivity of lesion detection at routine inspection. Twenty percent of meat processing plants that submitted samples during the study period submitted only one sample, and there were 13 meat processing plants that submitted no bTB-like lesions for confirmation which may in turn suggest a low sensitivity.

The risk of bTB confirmation varied between which disease control area an animal with a bTB-like lesion reported at routine carcass inspection was sent to slaughter from. The risk of bTB confirmation was highest in cattle with bTB-like lesions that were sent to slaughter from a

movement control area. Movement control areas correspond to the highest theoretical spatial risk of acquiring bTB from wildlife (OSPRI, 2021b). The association between disease control area and confirmation of bTB from bTB-like lesions is biologically plausible considering that movement control areas are where the risk of acquiring bTB from infected wildlife is clustered spatially, and the prevalence of known infected herds is also the highest. The area an animal is sent to slaughter from as a risk-factor for bTB confirmation is consistent with findings from other countries where wildlife vectors play a role in bTB transmission such as Great Britain and Northern Ireland (Pascual-Linaza et al., 2017; Shittu et al., 2013). This finding does not consider the lifetime history of cattle movements, but only the location cattle were sent to slaughter from. The large number of cattle movements typical of New Zealand's farming system, and imperfect movement records made it impractical to consider lifetime movement history in this analysis. Targeting animals sent to slaughter from a movement control area could be an enhancement to the slaughter surveillance system if there was a carcass-side mechanism of identifying such animals.

The study also demonstrated a higher risk of bTB confirmation in cattle with bTB-like lesions sent to slaughter from infected herds. This is consistent with findings from Ireland and Northern Ireland where a bTB restricted herd, or herds with a history of bTB infection are associated with an increased risk of bTB confirmation from bTB-like lesions reported at routine slaughter (Frankena et al., 2007; Male Here et al., 2022; Pascual-Linaza et al., 2017). A higher risk of bTB confirmation from bTB-like lesions from infected status herds indicates that routine meat inspection plays an important role in detecting bTB in herds where there is known bTB present despite that infected herds are exposed to a more rigorous ante-mortem testing regime (OSPRI, 2021b). Infected status herds or herds with a recent history of bTB infection may have bTB infected animals that have gone undetected by on-farm testing. Cattle during the early stage of bTB infection or with advanced generalised bTB infection can fail to return a positive skin test result despite being infected with bTB (de la Rua-Domenech et al., 2006; Pollock & Neill, 2002). Infection from wildlife vectors may have contributed to the higher risk of bTB confirmation from bTB-like lesions at routine carcass inspection from infected status herds. In New Zealand, infected status herds are concentrated within movement control areas which is where the risk of acquiring bTB from wildlife vectors is the highest (OSPRI, 2021b). In the dataset, nine out of 20 confirmed bTB positive bTB-like lesions were from a single infected herd. There may be herd-specific management aspects of this herd which is driving the significance of herd status as a risk-factor for bTB confirmation, but the analysis was limited by the number of positive outcomes in the dataset.

The finding that female is a risk-factor for confirmation of bTB from bTB-like lesions at routine carcass inspection is in agreement with findings of some reports (Frankena et al., 2007; Gonçalves et

al., 2022; Male Here et al., 2022; Olea-Popelka et al., 2012), but not others which found there was no difference in risk of bTB confirmation from bTB-like lesions between males and females (Pascual-Linaza et al., 2017). The implication of Sex as a risk-factor for bTB confirmation from bTB-like lesions may be limited. Sex is likely to be confounded by other variables such as age which was not included in the analysis due to missing data and therefore the effect of sex may be overestimated. Targeting females to enhance slaughter surveillance for bTB may not be an effective use of resources.

There was a difference in baseline risk of confirmation of bTB from bTB-like lesions submitted at routine carcass inspection between meat processing plants. Figure 3-2 representing the random intercepts for meat processing plant, displays these differences in baseline risk for the same level of all other covariates in the model. Plant-specific factors likely explain this observed variance between plants in the risk of bTB confirmation from bTB-like lesions submitted at routine carcass inspection. This was the case in Great Britain, Ireland, Northern Ireland and Spain (Male Here et al., 2022; Olea-Popelka et al., 2012; Pascual-Linaza et al., 2017; Pozo et al., 2021; Shittu et al., 2013). The confirmation rate of bTB-like lesions can be seen as a proxy for the inverse of the sensitivity of detection. As such, it may be relevant to further investigate plant-level factors that can influence this and attempt to standardise the performances of detection and bTB-like lesion submission across meat processing plants in NZ.

During the study period, 13% of newly infected herds were identified through routine carcass inspection (OSPRI Database), highlighting the importance of slaughter surveillance within the bTB surveillance system. This figure is lower than for Northern Ireland for example, where the proportion of new herd infection disclosed through routine carcass inspection ranged from 16.0% to 28.0% (Pascual-Linaza et al., 2017). In the present study, five (6.0%) of the new herd infections were detected through routine slaughter inspection in areas where cattle are not tested annually (Table 3-1) and may not have been subject to on-farm testing while infected, highlighting the critical role routine carcass inspection plays in identifying bTB infection in areas where the sensitivity of the livestock testing regime is lower.

Age was not considered for analysis due to the high proportion of animals of unknown age in the dataset. Age as risk-factors for bTB confirmation from bTB-like lesions reported at routine carcass inspection is variable in the literature (Male Here et al., 2022; Pascual-Linaza et al., 2017; Pozo et al., 2021), so an evaluation of age as a risk-factor for bTB confirmation may be of value in the future if improvements to lifetime data are observed.

In this study, the risk-factors for confirmation of bTB from bTB-like lesions reported at routine carcass inspection were evaluated. The risk-factors for bTB confirmation from bTB-like lesions

identified in this study may only apply to those with visible bTB-like lesions that were submitted for confirmation. Not all animals with bTB-like lesions or bTB infected animals would have had been detected or reported at routine carcass inspection. The stepwise factors influencing the sensitivity of slaughter surveillance for bTB include the probability that a bTB infected animal has grossly detectable lesions, the probability that a lesion is detected at routine carcass inspection, the probability that bTB lesions are submitted for diagnostic testing, and the sensitivity of diagnostic tests used for confirmation (Domingo et al., 2014; El Allaki et al., 2016; Garcia-Saenz et al., 2015; Sergeant et al., 2017). Submission of bTB-like lesions for confirmation was affected by disease control area, year and season, herd status, number of lifetime movements and was affected by unknown meat processor-specific factors as discussed in Chapter 2. The sensitivity of slaughter surveillance for bTB in New Zealand has been reported between 50% and 81% (Nugent, 2017; Pocięcha Jz, 1990), suggesting that a significant proportion of bTB infected animals are not detected or reported at routine carcass inspection. Targeting animals with risk-factors for bTB confirmation may not enhance the efficiency of the surveillance system as there may be other factors that are limiting the ability to detect or confirm the presence of bTB.

### 3.5 Conclusion

Herd status, sex, and the disease control area an animal was sent to slaughter from are risk-factors for confirmation of bTB from bTB-like lesions reported at routine carcass inspection. There is clustering of bTB within herds, and there are meat processor-specific factors that influence whether a bTB-like lesion is confirmed as positive. Routine carcass inspection plays a role in detecting new bTB infections and complimenting the on-farm bTB testing program. Identifying risk-factors for bTB confirmation from bTB-like lesions at routine carcass inspection may inform policy makers on potential modifications to enhance slaughter surveillance for bTB. This will enable efficient identification of bTB, and provide evidence of disease absence as New Zealand progresses towards total biological eradication of bTB.

## 4 General conclusion

This study evaluated risk-factors for the submission of bTB-like lesions at cattle routine carcass inspection, and risk-factors for the subsequent laboratory confirmation of submitted lesions, for the period 1 January 2019 to 31 October 2021. During the study period, there was one bTB-like lesion submitted for every 6378 carcasses inspected. bTB was confirmed in one bTB-like lesion for every 60 bTB-like lesions submitted. Risk-factors for bTB-like lesion submissions from cattle at routine carcass inspection (1.6) included being sent to slaughter from an infected or suspended status herd, having more than two lifetime movements and the year and season in which they were slaughtered. Risk-factors for confirmation of bTB from bTB-like lesions submitted by meat inspectors (3) included female cattle from infected status herds within movement control areas. The baseline risk of bTB-like lesion submission and confirmation of bTB also differed between meat processing plants. These findings indicate that meat processing plant-specific factors, not just animal-specific factors alone, influence whether a bTB-like lesion is submitted for testing or confirmed as bTB. The finding that meat processing plant factors were affecting bTB-like lesion submissions was consistent with other countries where the effectiveness and efficiency of slaughter surveillance for bTB has been evaluated (Pascual-Linaza et al., 2017; Pozo et al., 2021; Shittu et al., 2013). Given the significant variation in the bTB-like lesion submission rates between plants, a further investigation of plant-specific factors that are potentially influencing whether bTB-like lesions are submitted is warranted. Better understanding of factors influencing the probability for a meat processing plant to detect and submit bTB-like lesions would enable more tailored policy making to improve the overall submission rate.

Limitations of this research included the small dataset of bTB-like lesions confirmed as bTB positive ( $n=20$ ) compared to the number of cattle slaughtered ( $n=7,646,655$ ). For the analysis of the confirmation rate, the outcome was rare and clustered by herds and meat plants, probably resulting in a lack of representativity. Another constraint of the research was the limited number of relevant animal-level and herd-level variables available for inclusion in the analysis, such as age and lifetime history of bTB testing. Data pertaining to individual animals and herd were spread over two different databases that were poorly aligned which meant generalisations and assumptions such as herd status and number of movements were required to include those variables in the analysis. Integration of databases containing animal and herd level data and a requirement to record additional animal-level information would enable a more detailed analysis in the future. Considerations for enhancing slaughter surveillance for bTB at the animal-level or herd-level should be considered with these limitations in mind.

The emphasis on slaughter surveillance for the control of bTB in New Zealand is growing (OSPRI, 2021b). The information presented in this thesis represents a first step for the overall evaluation of bTB slaughter surveillance as fit-for-purpose to support bTB eradication. Even after adjusting for animal specific risk-factors, the submission of bTB-like lesions and confirmation of bTB varied significantly between meat processing plants. A complete evaluation of slaughter surveillance for the detection of bTB in New Zealand is recommended, including participation, coverage, and representativeness of slaughter surveillance, and further work on accuracy of detection at meat processing plants specifically. These attributes are essential for animal health surveillance systems where the primary purpose of surveillance is case detection to facilitate control or providing evidence of disease absence. A complete evaluation, based on this research, is warranted to determine required outputs and metrics for effective slaughter surveillance of bTB, and identify key areas for enhancement.

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