Time and space analysis of abattoir monitoring data of Johne's disease in New Zealand

A thesis presented

in partial fulfilment of the requirements

for the degree of Master of Veterinary Studies

at Massey University

Jun Hee Han

Institute of Veterinary, Animal and Biomedical Sciences

Massey University

Palmerston North, New Zealand

2016

(Submitted 14 March 2016)

Institute of Veterinary, Animal and Biomedical Sciences

Massey University

Palmerston North, New Zealand

2016

Dedicated to my lover Geun Young

for enduring all the pain caused by my intellectual greed,

and to my grandma who rests in heaven peacefully

Acknowledgments

I would like to express my sincere gratitude and thankfulness to my main supervisor, Cord Heuer. When this study was drifting in uncharted ocean, he was a lighthouse and his guidance was a ray of light to illuminate a way to go forward in the world of epidemiology. It has been an absolute honour for me being under his supervision.

I would like to extend my gratitude to my co-supervisor, Peter Wilson. Were it not for his general advice on deer industry in New Zealand, I would not acquire any insight to apply advanced statistical knowledge on my research.

I would also like to thank the lecturers, Carolyn Gates, Sara Rosanowski, Naomi Cogger, Jackie Benschop, and Mark Stevenson, in EpiCenre, IVABS, both in past and present. My appreciation also goes to staff members of EpiCentre, Chrisitne Cunningham, Simon Verschaffelt (Frisbee!!), and Tim Carpenter. Their teaching and helping formed a profound basis of me becoming an epidemiologist.

Special thanks to EuTteum and EunMi, who helped me unconditionally settle down in this foreign country and focus on my research. I would like to thank fellow EpiCentre students who showed me such an energetic enthusiasm on both life and study.

Finally, I would like to thank my family and fiancé. To my father and mother, I could have not achieved this much without your spiritual and financial support. It is entirely your loves and

devotions which carved me to stand here today. To my love, thank you for enduring the pain that you suffered because of my stubbornness.

Nomenclature

ACF	Autocorrelation Function
AIC	Akaike Information Criterion
ARIMA	Autoregressive Integrated Moving Average
ARMA	Autoregressive Moving Average
AUC	Area Under the Curve
CV	Coefficient of Variation
DSP	Deer Slaughter Premises
EML	Enlarged Mesenteric Lymph node
JD	Johne's Disease
JML	Johne's Management Limited
LEP	Longitudinal Enlarged mesenteric lymph node Prevalence
MAP	*Mycobacterium avium* subspecies *paratuberculosis*
PACF	Partial Autocorrelation Function
SARIMA	Seasonal Autoregressive Integrated Moving Average

Contents

List of Figures

List of Tables

CHAPTER 1

Introduction

1.1 Deer Industry in New Zealand

Deer has been domesticated in New Zealand since 1970s with the emendation of regulation to designate deer as a farming animal from the pest (Pollard, 1993). Since the establishment of deer industry, New Zealand has become one of the world leading countries for deer farming with annually producing over 20,000 and 400 tonnes of venison and velvet, respectively (DINZ, 2015). In early of 1990s, the estimated number of deer in New Zealand was over 1 million from more than 6,000 farms, and it increased up to 1.7 million deer until the peak of 2004 (Annonymous, 2005; Geoffrey W. de Lisle, Yates, & Collins, 1993). However, deer industry in New Zealand has been in downturn after 2004, and current total number of deer is estimated as 0.9 million (MacPherson, 2014). Also, the number of deer farm has reduced and is estimated less than 2,000 in 2014, however, the accurate figure is unknown because of the lack of monitoring or tracking system for entire deer farm.

1.2 Johne's disease in deer in New Zealand

Johne's disease (or Paratuberculosis), caused by infection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is granulomatous enteritis inducing diarrhoea, weight loss and even death in

ruminant (Collins, 2003). After ingestion of MAP, the agent is absorbed via ileo-jejunal epithelium and migrates to reginal lymph node captured by macrophage. As MAP can avoid immune mechanism of the infected host, it proliferates in lymph nodes causing enlargement of mesenteric lymph node with or without necrosis, which is predominant pathological symptom in deer (Jamie C. Hunnam, 2011). After the first diagnosis of Johne's disease in deer was reported in New Zealand in 1979 (Gumbrell, 1986), a series of Johne's disease cases in deer were published, confirming the endemic status of the disease throughout the country. At the beginning, Johne's disease in deer in New Zealand was mainly detected as a by-product of national tuberculosis surveillance programme. According to de Lisle et al. (Geoffrey W. de Lisle et al., 1993), 21 MAP isolates were found from the lymph node of farmed deer with positive to tuberculin skin test from 1970 to 1991. The same research team also reported the steadily increasing trend of MAP isolation, resulting in isolating MAP from 390 farmed deer in 2004 (Geoffrey W. de Lisle, Cannon, Yates, & Collins, 2005). Stringer et al. (2013) conducted a study to describe the population based national prevalence of Johne's disease by analysing normal mesenteric lymph nodes from abattoirs in the country. The study suggested that 59 % of deer herd were infected by MAP with 67 % individual deer being infected in those herds. Using the results of previous study as prior parameters, Verdugo et al. (2014) surveyed 97 deer herds by pooled culture and ELISA testing, and conducted a Bayesian estimation to calculate the national prevalence of Johne's disease, resulting in herd-level true prevalence at 46 % with significant difference between the North Island and the South Island (33 % vs. 54 %). However, there have been no attempts so far to describe the longitudinal pattern of disease which is continuously changing over time.

1.3 Johne's disease surveillance system

Johne's Management Limited (JML) is a surveillance programme funded by industry stakeholders for monitoring Johne's disease of deer in New Zealand. To follow the trend of the disease, information of every slaughtered deer has been recorded at Deer Slaughter Premises (DSP) since December of 2006. The information is mainly focused on the size of mesenteric lymph nodes, along with the age and weight of carcasses, farm identifier and farm's geographical coordinates. During the

slaughter process, meat inspector visually measures the size of mesenteric lymph node, and marks a deer as abnormal if a circumference of mesenteric lymph node is over 55 mm. Enlarged mesenteric lymph node (EML) over 55 mm is presumed to be an indicator of MAP infection which might be regarded as a sign of subclinical Johne's disease, because most deer with overt clinical signs of Johne's disease are removed before slaughtering. Jaimie C. Hunnam et al. (2013) reported that the prevalence of MAP culture-positve mesenteric lymph node among EML classified by meat workers was 92.2 %. However, according to Stringer et al. (2013), 45 % of all carcasses without EML were also MAP culture positive. Hence, EML is certainly not a sensitive indicator of MAP infection, but may indicate an effect of subclinical Johne's disease and be used as a signal for the farms being infected by MAP. EML may also appear in the absence of MAP and Johne's disease (e.g. Tuberculosis or Yersiniosis (Jerrett, Slee, & Robertson, 1990; Robinson, Phillips, Stevens, & Storm, 1989)). This would give rise to a lack of specificity of EML, causing false indication of MAP/Johne's disease affecting a herd when it was actually not present. Although EML may have merit for indicating a Johne's disease problem, a surveillance programme for the disease using EML may be considered as syndromic surveillance as it lacks diagnostic accuracy.

The EML based surveillance systems send notification letters to deer suppliers flagging a possible Johne's disease problem if at least one carcass in the mob was EML positive. Notified deer farmers are encouraged to reduce the prevalence of the disease. As mentioned above, visual EML inspection at slaughter may be typical but are not pathognomonic for Johne's disease, so it lacks diagnostic accuracy. It indicates the limit of the JML programme that the notification letter to suppliers could have wrong information caused by false positives. No evidence is currently available about the association between EML at slaughter and Johne's disease incidence on the farm of origin. To improve the abattoir monitoring and notification system, inaccuracy of the JML data should be removed, so as to identify the truly Johne's disease affected farms that possibly harbouring super shedders of MAP.

1.4 Dissertation Aim and Structure

This thesis is comprised of four chapters. The material presented in Chapter 2 is to provide an overview on application of syndromic surveillance in veterinary field, and concepts of time series analysis and space-time scan statistics that applied in the field using surveillance data. Chapter 3 is intended for descriptive purpose. Even though the overall number of deer in New Zealand is estimated and updated annually, there is no accurate figure for the number of deer farms in this country. Also, no studies have been conducted to describe the demographic characteristics of deer industry or to identify "commercial" farms constantly submitting venison to the market. So Chapter 3 describes the demographics of the deer industry in New Zealand using Johne's disease surveillance data. Among the "commercial" farms defined in Chapter 3, farms with a consistently high level of MAP infection are identified using a novel statistical approach by evaluating the prevalence of EML in Chapter 4. Chapter 5 provides the analysis on space-time cluster of commercial deer farms in terms of EML occurrence using space-time scan statistic.

CHAPTER 2

Literature review of the analysis of rich surveillance

2.1. Syndromic surveillance

Syndromic surveillance is a novel way of monitoring health indicators before the definitive diagnosis is made (Mandl et al., 2004). By seeking for the change in individual or population behaviour information, such as over the counter medicine sales or school absenteeism, an abnormal pattern or signal of possible disease outbreak can be captured in early stage. As development of syndromic surveillance was mainly driven by the agitation of bioterrorism after 9/11, syndromic surveillance in veterinary medicine also has relatively short history of establishment. Dórea, Sanchez, and Revie (2011) reviewed the application of syndromic surveillance in veterinary field, with reporting a variety of data sources, including management data, laboratory data, abattoir data, and zoological data, for detecting disease aberration. Among variable sources, abattoir data is a unique source for surveillance that only achievable in veterinary field. Abattoir data, compared with other sources, have advantages in; 1) Increasing the chance of detecting disease indicators, such as enlargement or necrosis of internal organs, and 2) Avoiding animal ethics issues that need to be approved for collecting blood or tissue samples in ante-mortem inspection. Application of abattoir data in syndromic surveillance can be identified from multiple sources. Dupuy et al. (2013) investigated a list of implemented syndromic surveillance system under the umbrella of "Triple-S project" in Europe using survey and literature review. According to the

research team, there were nine surveillance systems from six Europe countries that using abattoir data for the purpose of general health monitoring and disease outbreak detection.

2.2. Time Series Analysis

Time series is a set of data collected for multiple times from each sample throughout the time (Diggle, 1990). Because the observations from each sample are correlated based on the adjacency in the time, classical statistical analysis that assuming the independence of each observation is no longer applicable. In order to analyse the time series data, time series analysis has been used, and it is based on the dependence among the observations in sequence.

2.2.1. Frequency Domain

Time series analysis has been frequently used in veterinary epidemiology and public health as well. The aim of time series analysis in those fields was mainly in describing the longitudinal pattern of illness in population, with partial attribution to prediction of the future trajectory of diseases as well. In 1986, Carpenter and Hird (1986) conducted a study to describe the longitudinal pattern of mycobacteriosis in pigs from 1977 to 1981 using the Serfling method (Serfling, 1963). The Serfling method, also called as spectral analysis, is one way of time series analysis using the perspective of frequency domain to describe the longitudinal data. By using the combination of trigonometric function (e.g. sine and cosine), spectral analysis can describe the time series in mathematical way. Since then, a variety of research topics, ranging from the production of animal to the incidence of zoonosis, has been covered using spectral analysis in veterinary epidemiology or public health field (Jore et al., 2010; Sargeant, Shoukri, Martin, Leslie, & Lissemore, 1998). Another domain of time series analysis is based on the linear regression, with using the previous and current observation as predictor and outcome, respectively.

2.2.2. Autoregressive Moving average

One of the analyses in regression domain is Autoregressive Moving average (ARMA) model, accommodating the correlation between observations to the model by incorporating auto-regression of past values and partial averaging of prior errors (Shumway & Stoffer, 2010). In other words, the ARMA model describes the time series as a combination of lagged terms on the series itself and the lagged terms on the white noise (e.g. Gaussian error). General formulation of ARMA model, $ARMA\ (p, q)$, for stationary time series, y_t, can be shown as below:

$$y_t = \emptyset_1 y_{t-1} + \emptyset_2 y_{t-2} + \cdots + \emptyset_p y_{t-p} + \epsilon_t - \theta_1 \epsilon_{t-1} - \theta_2 \epsilon_{t-2} - \cdots - \theta_q \epsilon_{t-q} \quad (2.\ 1)$$

, where the mean of y_t is zero, $\emptyset$ and θ respectively indicate autoregressive and moving average operators, p is the autoregressive order, q is the moving average order, and ϵ_t is white noise. White noise is a random variable that having zero mean and constant variance with showing no correlation between its values (Becketti, 2013). In order to simplify the formula above, backshift operator, B, can be used. Previous observation of the time series by k period can be expressed as B^k, such that $B^k y_t = y_{t-k}$. Using backshift operator, equation (2. 1) can be shown as:

$$y_t - \emptyset_1 B y_t - \emptyset_2 B^2 y_t - \cdots - \emptyset_p B^p y_t = \epsilon_t - \theta_1 B \epsilon_t - \theta_2 B^2 \epsilon_t - \cdots - \theta_q B^q \epsilon_t \quad (2.\ 2)$$

, therefore:

$$(1 - \emptyset_1 B - \emptyset_2 B^2 - \cdots - \emptyset_p B^p) y_t = (1 - \theta_1 B - \theta_2 B^2 - \cdots - \theta_q B^q) \epsilon_t \quad (2.\ 3)$$

Formula (2. 3) can be more simplified as:

$$\emptyset_p(B) y_t = \theta_q(B) \epsilon_t \quad (2.\ 4)$$

The orders of lag for autoregressive and moving average parts are determined based on the significant values in partial autocorrelation function (PACF) and autocorrelation function (ACF), respectively. Interestingly, ARMA model has not been widely used in veterinary epidemiology. The

first appearance of researches using ARMA model in veterinary epidemiology was in 1990's, describing the longitudinal prevalence of fasciolosis and *Ascaris suum* infection in condemned ruminants and pigs, respectively, using data retrieved from abattoir (Goodall, McLoughlin, Menzies, & Mcllroy, 1991; McIlroy, Goodall, Stewart, Taylor, & McCracken, 1990). More recent research was conducted by Benschop, Stevenson, Dahl, Morris, and French (2008) that using ARMA model to fit the Salmonellosis surveillance data from Denmark.

2.2.3. ARIMA and SARIMA

ARMA model can be extended to Autoregressive Integrated Moving average model (ARIMA) or additive/multiplicative Seasonal Autoregressive Integrated Moving average (SARIMA) model. ARIMA model, $ARIMA\ (p, d, q)$, can be used if the series is not stationary. In ARIMA, the time series should be differenced by d order firstly to achieve the stationarity, then analysed as the same method with ARMA model. More advanced extension is SARIMA model that incorporates a seasonal fluctuation of the series by inserting additional differencing on the series. Multiplicative SARIMA model can be expressed as $SARIMA\ (p, d, q) \times\ (P, D, Q)_s$, where P is a seasonal autoregressive order, Q is a seasonal moving average order, subscript s is the length of seasonal period, and D is the order of seasonal differencing. For instance, s is 12 if the series was observed in every month and had annually repeating pattern. Equation of multiplicative SARIMA model based on the formula (2. 4) is shown as below:

$$\emptyset_p(B)\emptyset_P(B^s)z_t = \theta_q(B)\theta_Q(B^s)\epsilon_t \qquad (2.\ 5)$$

, where

$$z_t = \nabla_s^D \nabla^d y_t$$

, where ∇ indicates the differencing.

2.2.4. Time series analysis and Syndromic surveillance

For the syndromic surveillance that dealing with longitudinal data, time series analysis is applied in order to reduce the noise and detect the anomaly. In this case, fitted or estimated value from the time series analysis is working as a baseline, and any observation over the baseline is defined as abnormal status. Several studies have been published for time series analysis in terms of establishing the baseline in syndromic surveillance. Tsui, Wagner, Dato, and Chang (2001) conducted a study to detect the early signature of influenza epidemic in United States. In the study, the research team used Serfling method to fit the serial observations of influenza, then applied upper limit of 95 % confidence interval of the fitted value repeatedly to remove the outlier. When there was no observation being exceeding the upper confidence limit, it was used as a baseline to compare and identify the signal of outbreak. Few years later, one statistical study was published to suggest a guideline on a way to fit the time series as a baseline for establishing the automated biosurveillance system (Burkom & Murphy, 2007). According to the study, historical disease pattern can be estimated by the regression models, ARIMA model frameworks, or exponentially weighted moving average (e.g. Holt-Winter smoother), and the estimated value can be used for comparison. More advanced times series models, such as SARIMA, can be used for the establishment of baseline in surveillance system, especially when the longitudinal data are not able to be explained by those simple methods. Even though it's higher performance in fitness with data of SARIMA model, however, the advanced model is rarely used in syndromic surveillance. This is because the syndromic surveillance is generally aiming for early detection of disease outbreak with automated manner. As time series model becoming complex, automated system is hardly achievable because the parameters of the model should be updated manually along with the new data being uploaded.

2.3. Space-time Cluster

One of the goals of retrospective analysis of data that acquired from the surveillance system is to describe temporal and/or spatio-temporal pattern through statistical modelling (Höhle, Paul, & Held,

2009). This is also true even the surveillance system is targeting the syndromes of specific diseases. However, in order to describe the pattern and define the cluster of disease based on the syndromes, removing noise that embedded in the data should be preceded. After proper "sieving" process of data, longitudinal data can be analysed for disease cluster in space and time, if the data contains geographical information of disease. Kulldorff (2001) suggested a space-time scan statistic, which is a statistical method to detect space-time clustering of disease. The study described spatial clusters of disease in a certain point of time as circular windows with varying radii, of which each circle can contain up to 50 % of the population at risk. As a large number of circles can be created with each of them representing a spatial cluster, those circles can be overlapped. Then, the two-dimensional circle is extended into cylinder in three dimensions, with the base and height representing the space and time, respectively. The number of disease events in interest assumed to be either Poisson or Bernoulli distributed, each of distribution respectively applying in case of the observation is recorded as summed figure (e.g. positive cases in certain people-time at risk), or individual level (e.g. positive vs. negative). The study defined that the disease is clustered in space and time if the observed number of events in a cylinder is larger than expected. Null hypothesis of the test is no clustering in space and time, and Poisson generalized likelihood ratio that a cylinder having an outbreak can be shown as:

$$\left(\frac{c_A}{\mu_A}\right)^{c_A}\left(\frac{C-c_A}{C-\mu_A}\right)^{(C-c_A)}$$

, where c_A is the number of observed events in cylinder A, μ_A is the expected number of events in cylinder A based on the null hypothesis, and C is the total number of observed events during the study period in study region. The likelihood ratios are inspected for cylinders with various sizes of base and height, and as a lot of cylinders with different sizes being obtained and jointly covering the study region the cylinder with the maximum likelihood ratio is selected as the most likely cluster. Statistical significance of the clusters with the maximum likelihood ratio can be estimated by Monte Carlo simulation (Kulldorff, 2001). Conceptual illustration of space-time cluster is presented in Figure 2. 1.

After the development of free software, SaTScan™ (Kulldorff, 2015), that able to conduct Kulldorff's space-time scan statistic, epidemiologic researches that describing space-time cluster of disease using the statistics have been actively published. In 2010, Gautam, Guptill, Wu, Potter, and Moore (2010) conducted a study to determine if seropositive to Leptospirosis test in dogs was clustered in space and time using longitudinal data of 8 years. Martínez-López, Perez, and Sánchez-Vizcaíno (2011) conducted a study identifying the horse premises at high risk of introducing African horse sickness virus based on a space-time analysis of the cluster of *Culicoides* spp. mosquitoes. Although data acquired from the syndromic surveillance are also applicable for Kulldorff's space-time scan statistic, no research has been studied to detect space-time clustering using those type of data. However, as mentioned above, adjustment of data would be necessary in order to analyse the space-time clustering using syndromic data owing to the nature that the data containing a lot of noise.

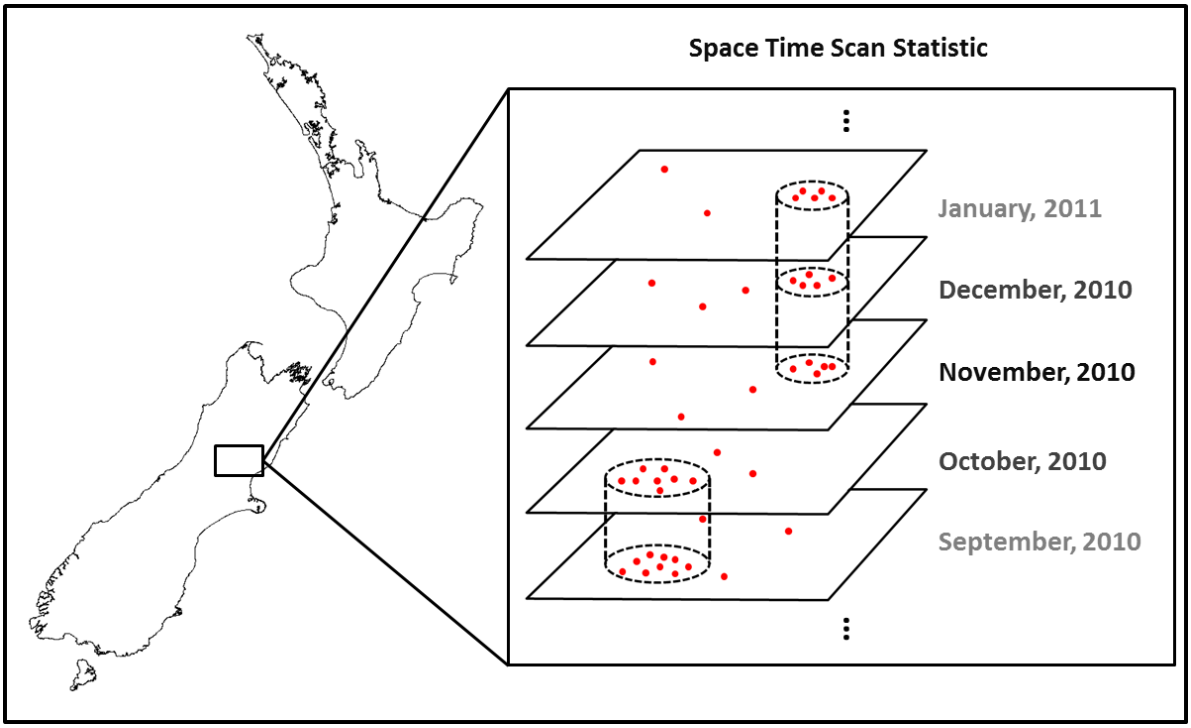

Figure 2. 1. Illustration of concept for space-time clusters. Red dot indicates the location of disease outbreak (e.g. farm, hospital), and each cluster is recognized as cylinder shape. The most likely cluster is selected based on the maximum likelihood ratio, and statistical significance is estimated by the Monte Carlo simulation.

CHAPTER 3

Demographic changes of deer industry in New Zealand from 2007 to 2014 using Johne's disease surveillance data

3.1. Introduction

Since the 1970s of domestication of deer in New Zealand for the first time, deer industry in this country has become one of the important business with annually producing over 20,000 and 400 tonnes of venison and velvet, respectively (DINZ, 2015; Pollard, 1993). In early of 1990s, the estimated number of deer in New Zealand was over 1 million from more than 6,000 farms, and it increased up to 1.7 million deer until the peak of 2004 (Annonymous, 2005; Geoffrey W. de Lisle et al., 1993). However, deer industry in New Zealand has been in downturn after 2004, and current total number of deer is estimated as 0.9 million (MacPherson, 2014).

Even though the number of deer in this country has been updated annually, the accurate demographic characteristics, such as the number of deer farms or "commercial" deer farms that constantly submitting venison to the market, have not been described so far. Also, as those

characteristics change over time, description of demographic in longitudinal perspective should be conducted in order to overview the overall pattern.

In the late of 2006, Johne's Management Limited (JML) was established by industry stakeholders for monitoring Johne's disease of deer in New Zealand. JML conducts a surveillance programme to follow the trend of the disease by gathering information of every slaughtered deer from Deer Slaughter Premises (DSP). The information is mainly focused on the size of mesenteric lymph node, along with the age and weight of carcass, identifier and geographical coordinates of farms. During the slaughtering process, meat inspector visually measures the size of mesenteric lymph node, and marks a deer as abnormal if a circumference of mesenteric lymph node is over 55 mm. Enlarged mesenteric lymph node (EML) over 55 mm is presumed as a possible indicator of subclinical Johne's disease, and a supplier of deer receives a notification letter of possible Johne's disease problem if at least one carcass among the mob that sent to DSP was EML positive. It is a motivating strategy for producers to reduce the prevalence of the disease. However, as the JML programme collects general information of slaughtered deer and farms that deer were originated, data can be used to describe and analyse the demographic pattern of deer industry in New Zealand.

Therefore, the aims of this study were to 1) Describe the demographic characteristics of deer industry, such as the number of deer slaughtered, number of deer farms or commercial deer farms, and the mob size, in New Zealand between 2007 and 2014, using Johne's disease surveillance data, and 2) Cleanse and manipulate the data for further analysis, including description of longitudinal EML pattern or identification of farms at high level of EML occurrence.

3.2. Materials and methods

3.2.1. Data Integration

For this study, two datasets were retrieved from the JML; 1) Data recorded deer slaughtered from December 2006 to December 2012, and 2) Data recorded from July 2012 to January 2015. The first dataset comprised of age, sex of slaughtered deer, date of slaughtering, and whether the deer had EML.

It also contained the locational information on farm that deer slaughtered originated from, DSP identifier where the deer slaughtered, and destination of venison consumption (local or export). The second dataset had the similar information with additional value on the maturity of deer, coding whether the deer was young or mature. For the analysis, two data were concatenated into a single JML dataset. Before merging two data sets, observations of the second half of 2012 from two datasets were compared to prevent the overlapping of observation. If observations were presented in both datasets, only one observation was used. Also, observations with unrealistic carcass weight with less than 20 kg or more than 250 kg were removed, and data with missing value on farm identifier, sex, or age were discarded.

3.2.2. Age and Maturity

In this study, age of deer should be unified before any analysis, as there were different levels of age code (e.g. 2, 2+, 3-, 3, 3+, 4, 4+, etc.). So we decided to categorise the age of deer into a binary variable (young / adult) based on their age code. However, each DSP had its own coding of age. Therefore, artificial cut-off value of age code for maturity categorisation was set up. In order to create the binary age variable, DSPs were categorised into 3 groups based on their age coding system; 1) Single age code group, 2) Dichotomous age code group, and 3) Multinomial age code group. The values of age code of DSPs in the same age code group also varied. For instance, among dichotomous age coding DSPs, five DSPs categorised deer slaughtered as either 2 or 2+, while eight DSPs used either 3- or 3+. To standardise the age of deer, distribution of weight in each age code in different sex from each DSP was analysed. It was assumed that the distribution of carcass weight from young deer could not vary widely as they had limited time for growing. So deer with age code; 1) Having lower mean of carcass weight than the ones from the other age code, and/or 2) With coefficient of variation (CV) of weight being less than 0.16 for hinds and 0.20 stags, were categorised as young for deer slaughtered from dichotomous age code group DSPs unless the distribution of weight itself indicated the mixture of distribution (e.g. bimodal). If a distribution of lower age code (e.g. 3- between 3- and 3+) suggested the mixture of deer from different age (e.g. large CV or bimodal), all deer from the age code were categorised as adult even though it had lower mean weight. For DSPs used multinomial age code, stags

were categorised based on the mean weight and CV (0.20). For hinds from those DSPs, however, the mean carcass weight and CV values were not distinctively different between young and old deer, as there was only a slight variation in weight. In this case, the maturity of those hinds was followed by the stag's maturity in the same age code. Observations from DSPs with single age code were deleted as it was impossible to compare the mean weight and CV. After the maturity value was given to each observation, two datasets were merged. Overall data integration process is illustrated in Figure 3. 1.

3.2.3. Farm categorisation

For the purpose of this study, deer suppliers were categorised into two binary groups based on their mob size of young deer in order to detect stable deer suppliers in industry. The two categories were; 1) Active status of business based on the mob shipping in the latest year in study period (2014). If a farm had not supplied any young deer in 2014, the farm was classified as inactive, otherwise it was active. 2) Stability of submission. A farm was considered as stable farm if; i) The farm supplied young deer to DSP every year after the first appearance of the farm on JML dataset, and ii) The farm supplied young deer to DSP more than two years. With the categorisation result of active status and stability of submission, farms were grouped further as either commercial or casual farm. If a farm was both active and stable, the farm was classified as commercial farm, otherwise it was casual farm. All the data manipulation and analysis in this study was performed using the PostgreSQL (The PostgreSQL Global Development Group, 2015) and the STATA (StataCorp, 2013). Graphs were plotted with the R statistical software using the package "ggplot2" (R Core Team, 2014).

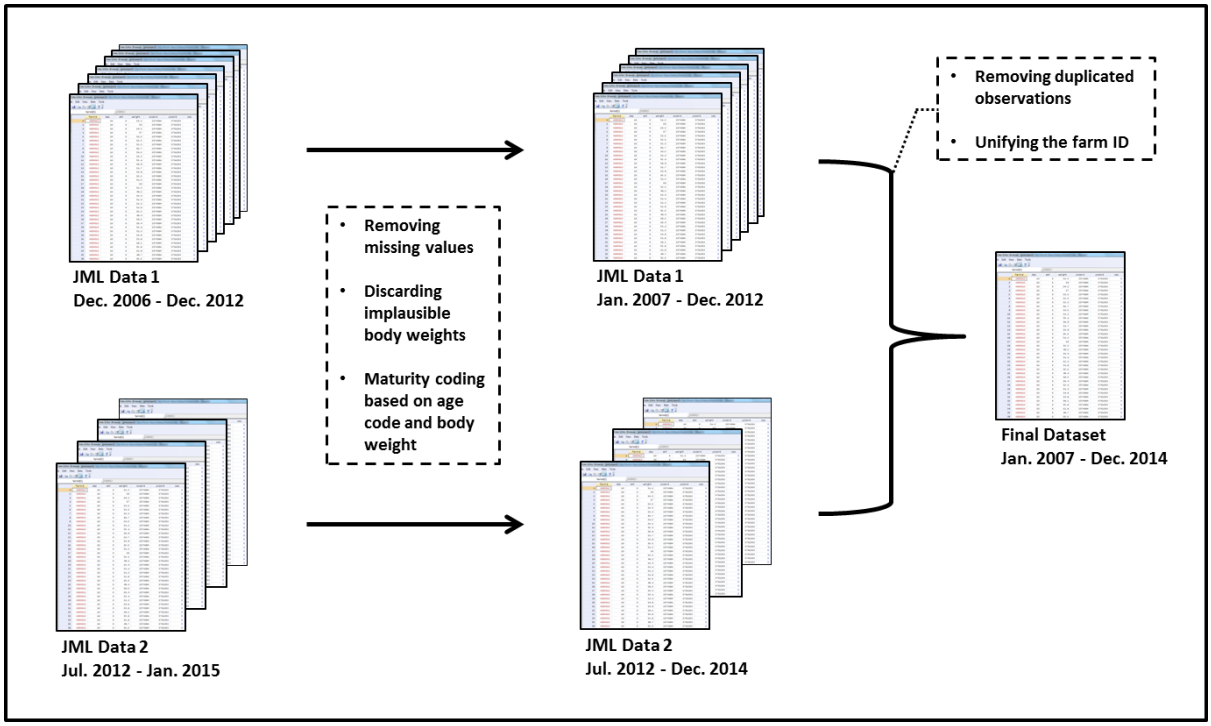

Figure 3. 1. Illustration of data integration process. The proportion of data in the final dataset is 98.9 % of entire raw data from two datasets.

3.3. Results

3.3.1. Data validation

Two datasets were concatenated into a single JML dataset. Before merging, observations of the second half of 2012 from the datasets were compared because of the overlapping. Based on the comparison, overall size of the observations in the last half of 2012 was larger in the second dataset than the first one. Nevertheless, some observations were missing in the second data while being presented in the first data. Those observations were extracted and updated into the second dataset, and updated second dataset was used for concatenation. Uniqueness of farm identifier was evaluated after merging, based on the coordinates of the farm. Only two farms shared the same geo-locational information (i.e. Two ids in one location), resulting in the records of two farms being united into a single farm.

For the analysis, the study period was narrowed from January 2007 to December 2014, as the records of December 2012 and January 2015 were partial information of deer slaughtered in each corresponding month. During the eight years, information of 3,587,264 slaughtered deer that originated from 4,201 farms (including missing value in farm identifier) were recorded at 21 DSPs. 36,923 observations were removed because of the extreme carcass weight or missing values. Additional 2,193 observations were discarded as they were slaughtered from DSPs using single age code, resulting in analysing 3,548,148 observations from 4,195 farms with 19 DSPs during the study period.

3.3.2. Demographics

- **General description**

The number of farm and deer slaughtered throughout the study period is illustrated in Table 3. 1. There was slight increasing in the number of farms and deer at the beginning, however they showed decreasing trend after 2008. The number of farms appeared on the JML dataset for the first time was decreasing from 2008 to 2014, while more number of farms showed the last appearance on JML dataset (right censored from Johne's disease surveillance system) during the same period.

Table 3. 1. Number of farm and deer slaughtered during the study period. The number of new and censored farms in 2007 and 2014, respectively, are omitted as the date of data starts and ends in those years.

Variable	**Year**							
	2007	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**
# Farms	2,860	2,874	2,522	2,227	2,186	2,054	1,861	1,760
# Deer slaughtered	528,998	572,949	455,747	381,653	405,767	402,821	403,220	396,993
# New farms	-	592	239	137	124	114	81	48
# Farms censored	392	499	358	242	273	305	366	-

#, the number of; -, not available

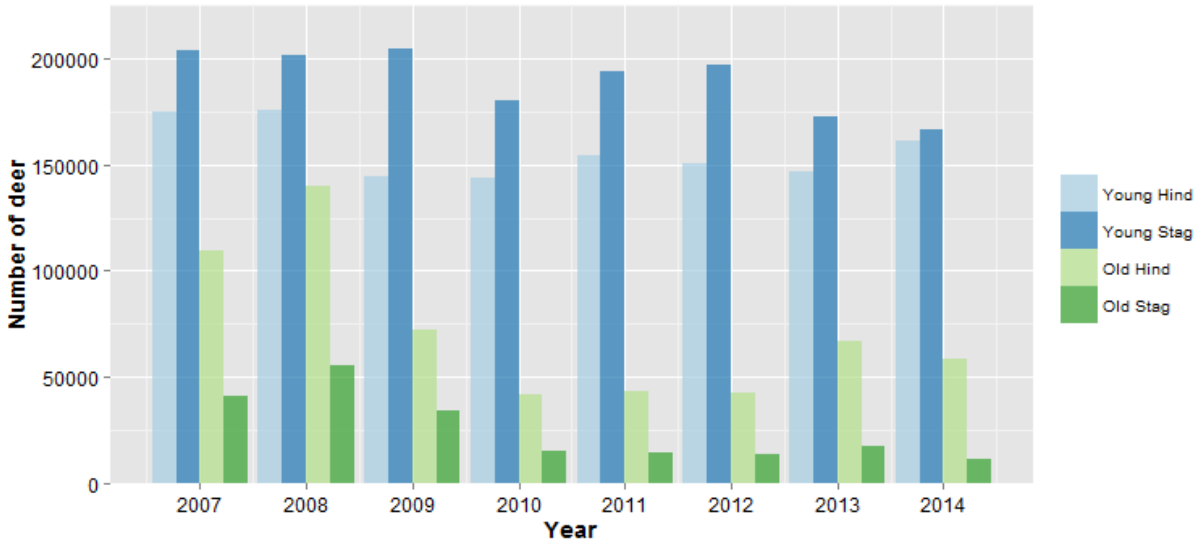

Figure 3. 2. Description of the number of deer slaughtered stratified by the sex and maturity during the study period.

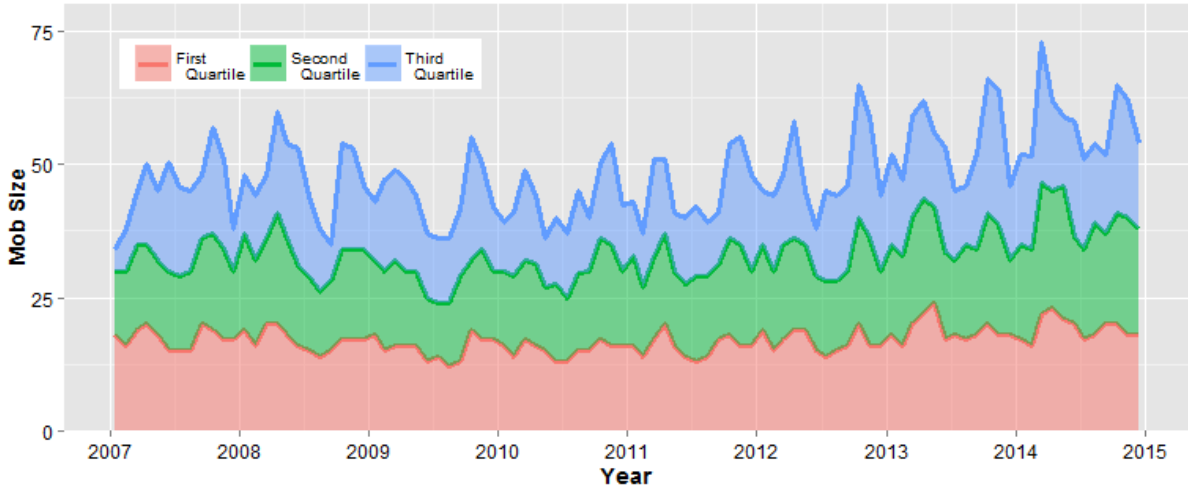

Figure 3. 3. Illustration of the mob size per month during the study period. Green line indicates the median, and red and blue lines are the first and third quartile of the mob size, respectively.

Among 21 DSPs, there were two single age code DSPs, 15 dichotomous age code DSPs, and four multinomial age code DSPs. For DSPs with dichotomous age codes, mean weight and CV were 55.3 kg, 0.17 for young stag, and 49.6 kg, 0.15 for young hind, respectively. For adult deer, mean carcass weight and its CV were 86.1 kg, 0.28, 54.3 kg, and 0.17, respectively, for stags and hinds from those DSPs. For multinomial age coding DSPs, mean weight of young and old stags were 56.1 kg and 89.4 kg, respectively. For hinds, mean carcass weight were 48.8 kg and 53.3 kg for young and adult deer, respectively. Mean CV of stags were 0.17 and 0.24 (young and old), whereas 0.16 and 0.24 (young and old) for hinds, respectively, for deer from those DSPs. Number of carcasses during the study period is described in Figure 3. 2 based on the sex and maturity. During the study period, 78.1 % of deer were labelled as young, of which the proportions of stags and having EML were 54.9 % and 0.9 %, respectively. In adult deer, the proportions of stag and deer with EML were respectively 26.1 % and 0.2 %.

Mob size, which is the aggregated number of carcasses from each farm in monthly interval, varied dynamically, ranging from one to sometimes more than 2,000, with right skewed distribution. Mob size showed seasonal fluctuation, repeating peaks in both summer and winter, and there was a slight increasing tendency in mob size after 2011. Median and interquartile range of the number of mob size in respect of time is plotted monthly in Figure 3. 3.

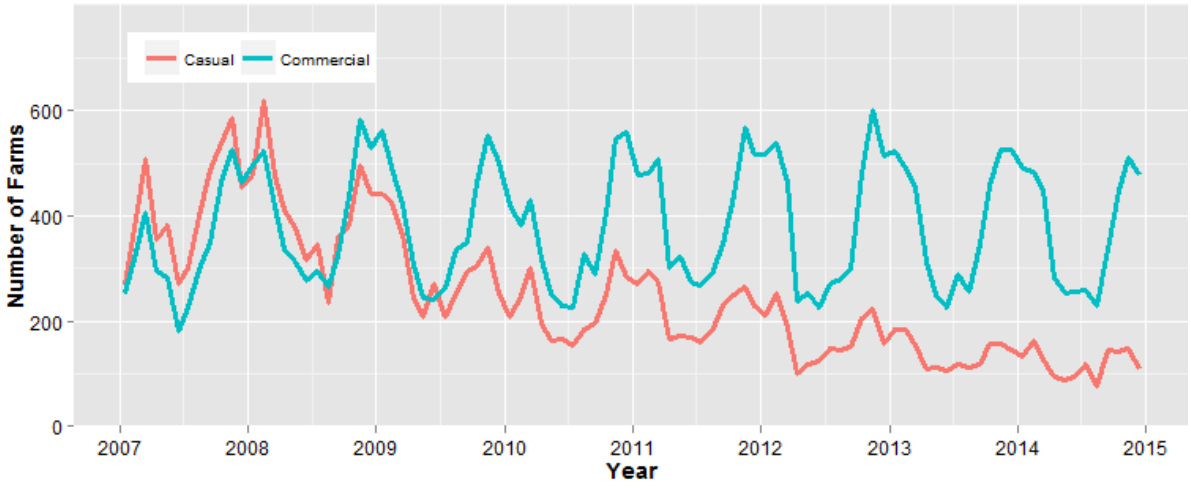

Figure 3. 4. Time series plot of the number of casual and commercial farms that submitted deer to deer slaughtering premises for venison production.

Figure 3. 5. Time series plot of the number of deer slaughtered from entire farms and commercial farms during the study period.

- **Commercial farm**

In the final dataset, 2,772,044 observations from 3,931 farms were young deer, and 264 farms submitted adult deer only to DSP during the study period. Among those 3,931 farms, 1,654 farms (42.1 %) submitted a mob in 2014, and 1,660 farms (42.2 %) submitted a mob more than two consecutive years since their first appearance on the Johne's disease surveillance data. Based on the criteria above, 1,060 among 4,195 farms (25.3 %) were defined as commercial farm, from which 2,530,360 deer were submitted to DSPs during the study period. The numbers of casual and commercial farm, and the number of deer from those farms are illustrated as time series plot in Figure 3. 4 and Figure 3. 5, respectively. The number of both casual and commercial farm showed regular fluctuation with the peak in summer and trough in winter. There was a decreasing trending of the number of casual farm, while the commercial farm remained constantly. The number of deer submitted to DSPs also showed the same seasonal pattern with the number of farm. Difference of the number of submitted deer between casual and commercial farms was larger in summer than winter, indicating casual farms tended not to submit deer in winter season.

3.4. Discussion

This study analysed the Johne's disease surveillance data that collected from DSPs in order to describe the demographic pattern of farmed deer in New Zealand. According to Jaimie C. Hunnam, Heuer, Stevenson, and Wilson (2009), the proportion of deer slaughtering that captured into Johne's disease surveillance system approached to 100 % after the mid of 2008. It indicates that the JML data that we used contained almost every farmed deer that killed in New Zealand. Therefore, it is totally plausible to assume that the demographic change on this study represents the demographic characteristics of New Zealand deer industry.

For categorisation of deer age, we used both the mean and coefficient of variation of weight for each age code stratified by year and sex. The criteria of CV of carcass weight for being categorised as adult stag and hind were 0.20 and 0.16, respectively. Those criteria were chosen based on the

distribution of weight in JML data itself and could not work universally throughout the different DSPs. However, the inspection of CV values was a tool for aiding the categorisation process based on the mean carcass weight, and, furthermore, there was no substantial difference of overall weight and CV values between DSPs with different age coding system in young and adult deer.

In this study, commercial farms were defined as being constantly submitting deer to DSP with the record in the most recent year. For the stable submission, farms should have a record of submission on the dataset for at least three consecutive years. However, the three-consecutive-year was an arbitrary chosen criterion. Another criterion of having the submission record in 2014 for being a commercial farm was also an arbitrary decision. However, the basic concept for being commercial was to exclude any farm that had one or more years of no-supply, indicating it was a casual supplier. This assumption is legitimate when considering the facts that; 1) Deer is a seasonal breeder that mating and calving around April and December, respectively, in southern hemisphere in every year, and 2) Most farms of venison production operate 12-month venison system, which gives optimum profitability and efficiency in New Zealand deer industry context (Barry, Wilson, & Kemp, 1999).

Mob size showed a slightly increasing trend with seasonal fluctuation. Although it varied dynamically, mob size increased in both summer and winter, generally. The large mob size in summer can be explained by the breeding pattern of deer in New Zealand, while peak in winter can represent deer removed from the farm for the efficient use of grass.

According to the result of this study, the number of casual farm showed decreasing trend. It could represent the diminishing trend of entire deer industry in New Zealand (Annonymous, 2005; MacPherson, 2014). It also could be an artificial effect caused by the definition of the commercial farm itself. This is because the farms that appeared on the JML data for the first time in later of the study period have more chance to be categorised as commercial farm than the farms appeared for the first time earlier. However, the former explanation seems more legitimate as the number of new farms that appeared on the dataset showed decreasing pattern (Table 3. 1). Another reason that the industry is diminishing is that the numbers of slaughtered young stags and hinds were becoming closer as time

goes by (Figure 3. 2), indicating that more young hinds were submitted for venison production, rather than remained for breeding stock.

3.5. Conclusion

This study described the demographic pattern of farmed deer in New Zealand by analysing the longitudinal JML data. We suggested a method to unify the age code using the carcass weight, and criteria for classifying suppliers as commercial or casual deer farmers. Even though this study focused merely on data cleaning / selection process and descriptive statistics, it was the first study to describe demographic characteristics of the New Zealand deer industry with a longitudinal perspective using abattoir data. Also, this study made the further analysis possible; such as describing the national level of EML in deer or detecting a cluster of EML positive deer.

CHAPTER 4

A novel approach to identify commercial deer farms with a high level of *Mycobacterium avium* subspecies *paratuberculosis* infection in New Zealand

4.1. Introduction

Johne's disease (or Paratuberculosis) in deer, caused by infection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a therapy resistant, granulomatous enteritis inducing diarrhoea, weight loss and ultimately death or culling (Collins, 2003). After ingestion, MAP is phagocytised by macrophages and transported to regional lymph nodes. It proliferates inside the lymph nodes by evading immune reaction of the host, resulting in tissue reaction, such as enlargement of mesenteric lymph node with or without necrosis, which is a predominant pathological symptom in deer (Jamie C. Hunnam, 2011). Since the first report of Johne's disease in deer in New Zealand (Gumbrell, 1986), a series of studies confirmed that the country has been in endemic status of the disease throughout the country (Geoffrey W. de Lisle et al., 2005; Geoffrey W. de Lisle et al., 1993; Stringer et al., 2013; Verdugo et al., 2014). In order to decrease the burden of the disease, Johne's Management Limited (JML) was established by the industry stakeholders. The aim of the programme is to monitor the level of Johne's disease in slaughtered farmed deer in New Zealand by collecting information of the size of mesenteric lymph node at Deer Slaughter Premises (DSP). Based on the research of Jaimie C. Hunnam et al. (2009),

an individual carcass is recorded having one or more enlarged mesenteric lymph nodes (EML) if the node circumference is over 55 mm. If at least one deer of a mob submitted to a DSP is EML positive, the deer supplier will receive a notification letter of possible Johne's disease infection in the farm, motivating producers to initiate control practices for reducing Johne's disease prevalence. A critical assumption, for which no evidence exists to date, is that EML positive mobs come from the farms where Johne's disease causes significant production loss.

For population health monitoring, abattoirs are data sources for syndromic surveillance in veterinary field (Dórea et al., 2011). Abattoir data, compared with other sources, have advantages in respect to applying on syndromic surveillance system as they have characteristics of; 1) Increasing the chance of detecting disease indicators, such as enlargement or necrosis of internal organs, and 2) Avoiding animal ethics issues that need to be approved for collecting blood or tissue samples in ante-mortem inspection. Several studies were conducted to describe the trend of disease index using syndromic surveillance data from the abattoirs. In 2012, one research team published studies describing the longitudinal pattern of hepatic and pneumonic lesions in slaughtered pigs in England (Sanchez-Vazquez, Nielen, Gunn, & Lewis, 2012a, 2012b). Vial and Reist (2014) conducted a study to describe trends of whole carcass condemnation rates in Swiss and to evaluate the capability of data using for early detection of any animal disease. The JML programme, which is collecting data at slaughter stage as well, can be categorised as syndromic surveillance with abattoir data, and data retrieved from the surveillance programme can capture the change of Johne's disease level in deer in New Zealand.

A critical issue about the JML surveillance system is that farmers are notified about the possibility that Johne's disease is may be a problem on their farm if one or more deer slaughtered among the mob is EML positive. As EML is not a definitive diagnosis for the Johne's disease incidence on farm, results of the system are subject to error, such as other illness or false positives Johne's disease at the farm. Especially when the prevalence of EML is low, it is likely to be a poor predictor of clinical Johne's disease on farm. An advisable initial approach may be that JML notifies farms that have a high prevalence of EML in their slaughter stock. This might increase the sensitivity of the system and decrease the burden of the disease more effectively. As the purpose of the JML surveillance system is

to intervene and control Johne's disease, detecting high shedding farms and animals may have merit for being better able to control MAP "super-shedders" and reduce Johne's disease incidence.

In the JML programme, tracing-back of surveillance findings (e.g. EML) to farms with a high level of the disease can be achieved by 'comparing' the pattern of EML in mobs with the normal 'baseline' EML pattern. One challenge in the JML programme as well as other syndromic surveillances is in defining the normal baseline, because it contains a lot of inherent variation. With the aim to reduce variability in normal patterns, several studies have suggested a variety of methods that mainly based on control chart or time series analysis (Burkom & Murphy, 2007; Serfling, 1963; Tsui et al., 2001; Yahav, Lotze, & Shmueli, 2011). Another more crucial challenge is that no attempts have been made to establish the statistical methods for comparing and identifying the surveillance unit with high level of the disease. Most of analysis using surveillance data has been mainly focused on detecting possible outbreak signatures at aggregated level (e.g. Analysing regional or national level outbreak when the surveillance unit is farm). As the purpose of this study was to identify commercial deer farms (e.g. surveillance unit) with a high level of MAP infection in New Zealand, there was a necessity to establish a statistical approach to compare and identify the deer farms with high level of the disease.

So the aims of this study were; 1) To describe the longitudinal EML pattern of deer in New Zealand, and 2) To develop a novel way for identifying likely super-shedders of MAP in deer by detecting outlier farms by comparison with the longitudinal pattern.

4.2. Materials and Methods

4.2.1 Data preparation

In this study, the JML data collected from 2007 to 2014 were used. Any observation with missing value or duplicated information was discarded. Then, Johne's disease surveillance data were limited to only include observations from commercial farms. A commercial farm was defined as a farm having submitted deer to deer slaughtering premises (DSP) more than two consecutive years. The extraction

process is described in the previous chapter. In this study, we decided to analyse only the young deer for estimating the prevalence of enlarged lymph node (EML), with the aim of decreasing false positives which could occur due to a higher risk of other diseases causing EML during the adult productive life. Therefore, observations of adult deer were removed. Also, in order to increase the precision and to prevent the overestimation of prevalence, observations from mob size less than 20 deer were excluded. Mob size was defined as the aggregated number of deer submitted to DSP from a farm in each calendar month. To describe the longitudinal pattern of young deer with EML, observations were aggregated by monthly intervals and plotted as a time series graph. The national prevalence of EML in young deer was calculated by dividing the number of young deer with EML by the number of young deer slaughtered during each month.

4.2.2 National EML prevalence

Two different models were established to describe the national EML prevalence. The first model used a multivariate linear regression, while the second model was multiplicative seasonal autoregressive integrated moving average (SARIMA) model.

- **Linear regression**

National EML prevalence was modelled using linear regression in monthly interval. Outcome of the regression was the EML prevalence in each month, and explanatory variables were year (e.g. 2007, 2008, and so on) and month (e.g. January, February, and so on). Partial F test was used to examine the significance level (P value < 0.05) of those variables. Standardised residual of the model containing only the significant covariates was inspected to examine the violation of homoscedasticity assumption. Linearity assumption was inspected using quantile-quantile plot. Regression was performed using the STATA (StataCorp, 2013).

- **Time series analysis**

SARIMA model is an extension of Autoregressive Moving average (ARMA) model, incorporating a seasonal fluctuation of the series by differencing the series by order of seasonal period (e.g. 12^{th} order differencing for monthly observed data). In order to conduct the SARIMA model, the time series should be stationary, indicating that the series should have a constant mean and variance. If the series is not stationary, the time series should be pre-processed by differencing or subtracting the general trend. First, in order to stabilize the series, the time series of national EML prevalence was de-trended by fitting polynomial regression. To conduct the polynomial regression, month was centred and polynomials of centred month were created. The highest degree of polynomial was estimated by the visualization of the overall time series. After conducting polynomial regression, the P value of the highest polynomial degree was inspected. If the P value was not significant (> 0.05), the model was re-fitted based on the polynomial regression with the highest order being one degree lower than the previous model. To identify the presence of seasonality on the de-trended national EML prevalence, seasonal sub-series plot was examined. Also, a periodogram was plotted to identify whether the frequency for seasonality was statistically significant. Significant frequency was defined by comparing lower confidence limit of spectral density of the period with spectral densities of other periods. Lower confidence limit of the spectral density was calculated using the chi-square distribution with the two degree of freedom (Shumway & Stoffer, 2010). If the lower confidence limit of a certain period was higher than that of any other periods' spectral density value, the period was defined as significant.

To fit the de-trended data with seasonality, multiplicative SARIMA (Seasonal Autoregressive Integrated Moving Average) model was established. The autocorrelation function (ACF) and the partial autocorrelation function (PACF) of de-trended national EML prevalence that differenced by the significant period were inspected to decide the orders of seasonal and non-seasonal AR and MA. After fitting the SARIMA model with the estimated parameters, the order with insignificant coefficient with alpha level 5 % was dropped out from the model manually in stepwise manner. To select the final model, Akaike information criterion (AIC) was assessed, and residual was inspected by cumulative periodogram and Ljung-Box test for the model diagnostics. Time series analysis was conducted using

the STATA (StataCorp, 2013), and the results were reported back into the R for displaying with "ggplot2" package (R Core Team, 2014).

4.2.3 Longitudinal EML Prevalence (LEP) Scoring system

For identification of the farms at higher than expected prevalence of EML in longitudinal perspective, longitudinal prevalence of EML in each farm during the study period was compared with the baseline prevalence acquired from the models. The baselines for comparison were estimated as below:

$$Baseline = Estimated\ prevalence_{model} + (3.09 \times S.E._{model})$$

, where 3.09 is the approximate value of the one-tail 99.9% point of the standardised normal distribution, and S.E. is the standard error of the model estimated prevalence calculated by the model residual. As the frequency of the mob submission to DSP in a year and the mob size in each submission for a farm varied, those differences were adjusted for the comparison. In order to adjust the mob size in each submission, the concept of statistical power for binomial distribution was applied. If a mob had a higher prevalence of EML than the baseline, a binomial distribution with mob size (n) was applied to this mob's observed number of EML. Then, a vertical line of the expected number of EML positive deer in the mob was plotted on the distribution. The expected number of deer with EML in the mob was calculated as the product of the baseline prevalence and the mob size. If the expected number was not an integer, it was rounded up. The area under the curve (AUC) of the binomial distribution above the vertical line indicated likelihood for the prevalence of EML in the mob being higher than the 99.9% upper limit of the model estimated prevalence at that point of time. The maximum score of each point was one. All available scores of the mobs of a given farm were summed and divided by the number of mobs supplied by the farm during entire period until the point of analysis. The total number of mobs also included mobs with EML prevalence lower than or same as the baseline or zero. The resulting mean score (i.e. LEP score) was designated to each farm to indicate the degree of prevalence of EML

since having participated in the JML surveillance programme. The process of calculating the LEP score for each farm is illustrated in Figure 4. 1. Based on the LEP score, farms were categorised into either low, moderate, or high level of MAP infection. A low level of MAP infection was defined as the LEP score being zero. Farms not included in low level were categorised as moderate level of MAP infection if they were within the third quartile of scores larger than zero (< 75% of scores that > 0). Farms in the remaining upper quartile were identified as a high level of MAP infection. To quantify the degree of agreement between scoring systems using two different baselines, Cohen's kappa test was conducted (Cohen, 1960). All the analysis related to LEP score was performed using the STATA (StataCorp, 2013).

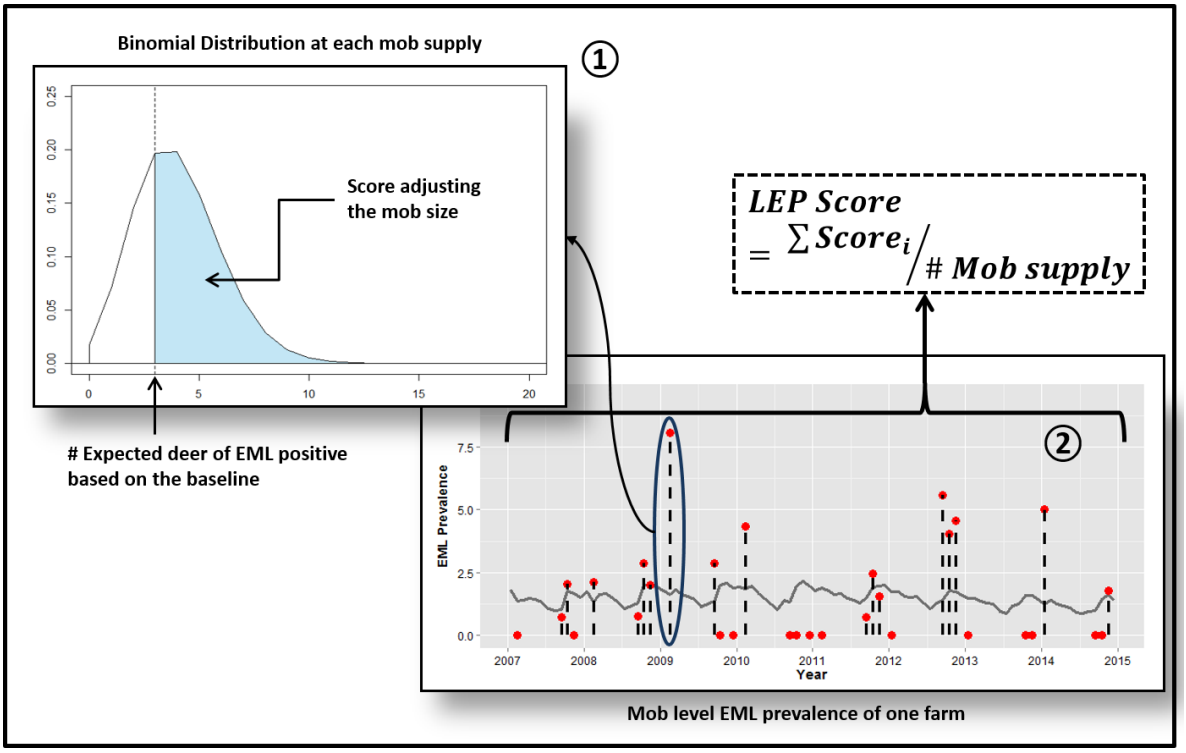

Figure 4. 1. Illustration of the process of calculating longitudinal enlarged mesenteric lymph node (EML) prevalence score. Right bottom graph describes the EML prevalence in every mob supply from one farm (red dot with dashed vertical line) along with the baseline (solid line). For each supply, score representing the EML prevalence in the mob exceeding the baseline was calculated based on the statistical power of the binomial distribution. If the prevalence in the mob was less than the baseline, the score on that point was zero. After all scores of every supply were calculated, the mean value was calculated for representing the overall degree of EML prevalence exceeding the baseline of the farm. # is the number of deer, and i and each red dot indicate the supply of mob in month from farm.

4.3. Results

4.3.1. Study population

The number of the deer during the study period was 2,092,451 from 1,017 farms, and it was the 58.9 % and 82.7 % of the raw data without missing values and the data from commercial farms, respectively. Proportion of the stag and the EML positive among the study population were 56.1 % and 1.0 %, respectively, and when stratified by sex, stags showed slightly higher proportion of EML positive than hinds (1.1 % vs. 0.8 %).

Mean mob submission rate was 3.1 times per year per farm during the study period, with the median being 2.6 times (Interquartile range from 1.5 to 4.4). Mob size per each submission showed right skewed distribution, with the mean and median being 94.0 and 55.0, respectively. The minimum and maximum numbers of EML positive deer in the mob were 0 and 11, respectively.

4.3.2. National EML prevalence

- **Linear regression**

The result of the multivariate linear regression is described in Table 4. 1. Partial F test of the explanatory variables in the multivariate linear regression showed both year and month are significantly associated with the prevalence of EML in each month. Adjusted R-squared value of the model was 0.74, indicating substantial proportion of the outcome was explained by the model. Though the distribution of residual in quantile-quantile plot showed some deviation from the 45° diagonal line, most of the standardised residuals were laying between -1.96 and 1.96 (not presented). Figure 4. 2 illustrates the national EML prevalence from 2007 to 2014 and fitted value of the model.

Table 4. 1. Results of the multivariate linear regression on national enlarged mesenteric lymph node prevalence in slaughtered from commercial farms.

Variables	Coefficient	Standard Error	P value	95 % Confidence Interval
Year			< 0.001	
2007	Reference			
2008	0.195	0.005		0.185 – 0.204
2009	0.293	0.005		0.284 – 0.302
2010	0.300	0.005		0.292 – 0.309
2011	0.366	0.004		0.358 – 0.375
2012	0.022	0.004		0.013 – 0.030
2013	-0.218	0.005		-0.226 – 0.209
2014	-0.120	0.005		-0.129 – 0.111
Month			< 0.001	
January	Reference			
February	0.035	0.005		0.026 – 0.045
March	-0.025	0.005		-0.034 – 0.015
April	-0.132	0.005		-0.143 – 0.122
May	-0.145	0.006		-0.156 – 0.134
June	-0.366	0.006		-0.378 – 0.354
July	-0.478	0.006		-0.490 – 0.466
August	-0.292	0.006		-0.304 – 0.281
September	-0.274	0.005		-0.285 – 0.264
October	0.084	0.005		0.075 – 0.093
November	0.263	0.004		0.254 – 0.272
December	0.163	0.005		0.154 – 0.172
Constant	0.910	0.005	< 0.001	0.901 – 0.920

Interpretation: National enlarged mesenteric lymph node prevalence in slaughtered commercial farmed deer in April 2012 is 0.8 % (0.910 - 0.132 + 0.022).

- **SARIMA model**

As overall trend of EML prevalence showed cubic curvature shape, third-order and second-order polynomial regressions were fitted into the data initially. As P value of coefficient of third-order polynomial was 0.15, second-order polynomial regression was applied for removing non-linear trend. Coefficient and P value of the second-order polynomial regression model are described in Table 4. 2.

To estimate the presence of seasonality, seasonal subseries plot and periodogram (Figure 4. 2) were visually examined. Among the three frequencies with distinctive spectral density in Figure 4. 3, only the lower limit of spectral density at frequency 1 was higher than any other spectral densities, resulting in only the 12 month being considered as the period of significant seasonality. ACF and PACF of 12^{th} order differenced EML prevalence suggested the parameter of non-seasonal and seasonal AR and MA as 1, 1, 1, and 1, respectively (Figure 4. 4). By removing covariates of P value being over 0.05 in backward stepwise manner, two SARIMA models, $(1, 0, 0) \times (0, 1, 1)_{12}$ and $(0, 0, 1) \times (0, 1, 1)_{12}$ were remained. Among them, SARIMA model with $(1, 0, 0) \times (0, 1, 1)_{12}$ was selected for describing the EML prevalence trend because of the lower AIC (-13.2 vs. -11.5). P value of Ljung-Box test for the model was 0.92 and cumulative periodogram of residuals were lying within the confidence interval, indicating that the model was valid. Coefficient and its P value of SARIMA model are described in Table 4. 2 and fitted value of SARIMA model is illustrated in Figure 4. 2 along with the national EML prevalence. Because of the differencing of 12^{th} order for adjusting the seasonal effect, the fitted value of the first year was not able to be predicted. As a result, the fitted value for the first year in the model was replaced to the original EML prevalence. The fitted values of both the polynomial regression and the SARIMA model were summed to calculate the model fitted value of overall trend of EML prevalence.

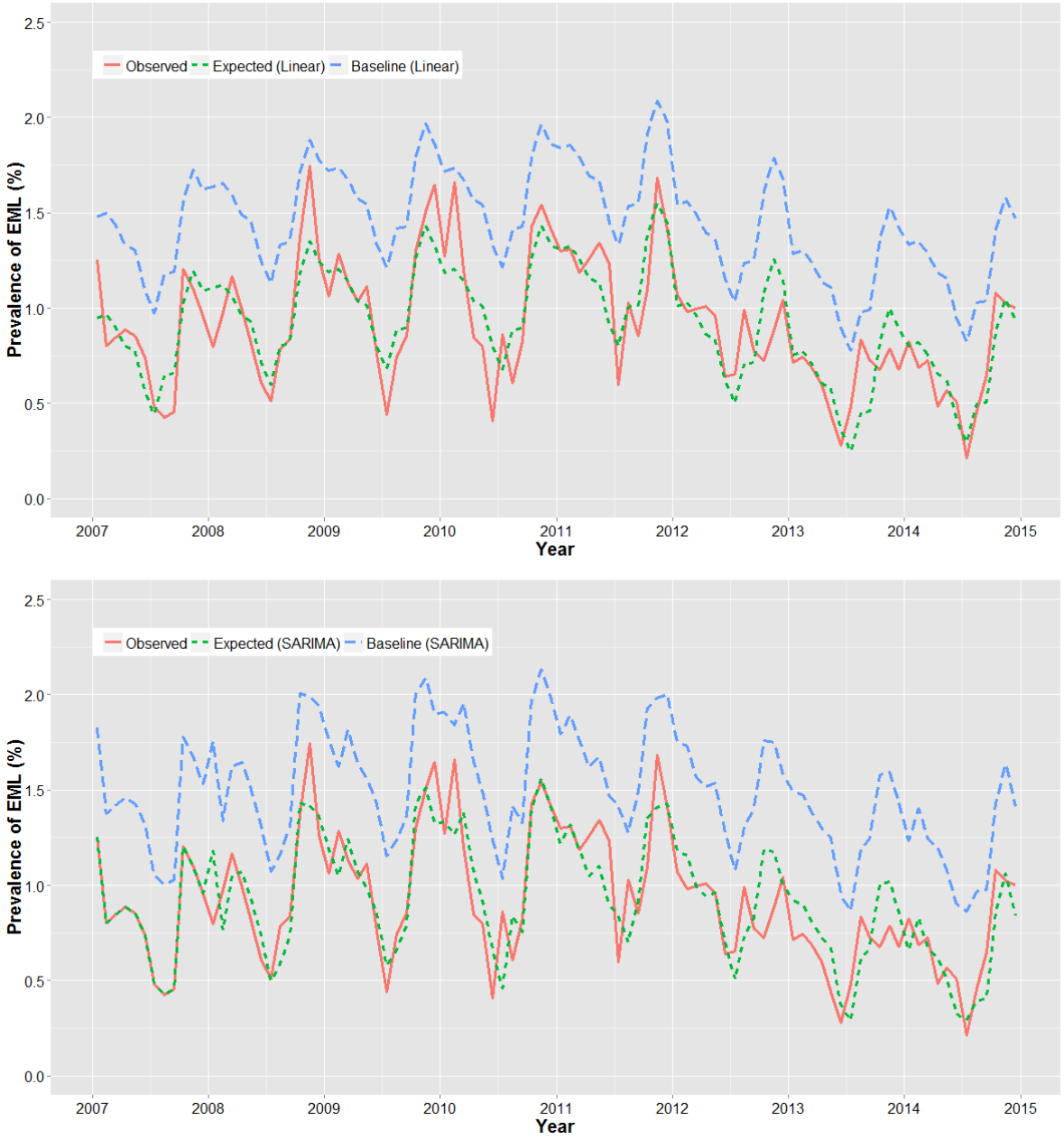

Figure 4. 2. Illustration of longitudinal prevalence of national enlarged mesenteric lymph node and its predicted values based on multivariate linear regression (Top) and multiplicative seasonal autoregressive integrated moving average (Bottom) models. Baselines of each model are estimated with the alpha level of 0.001.

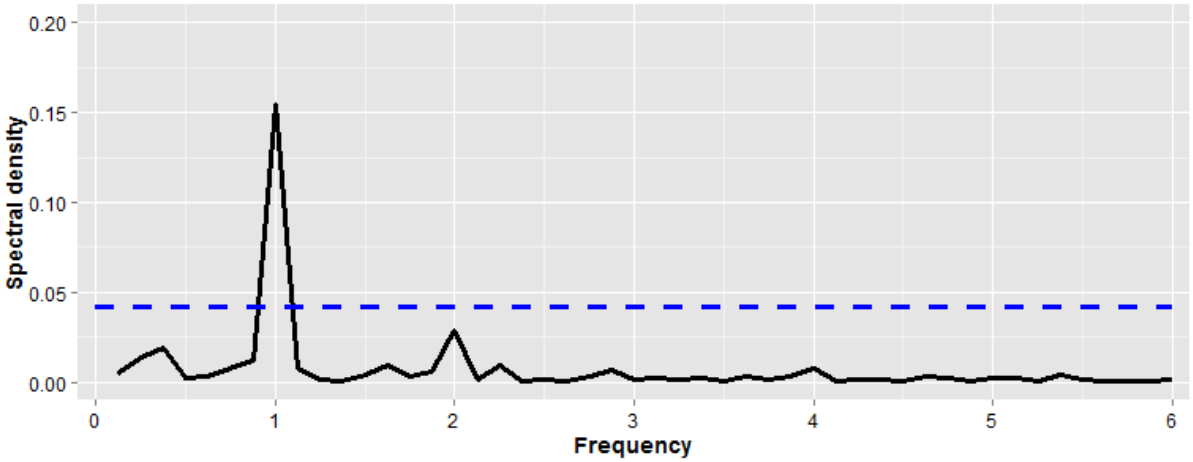

Figure 4. 3. Periodogram of polynomial regression adjusted national enlarged mesenteric lymph node prevalence. Peaks at frequency 1 and 2 indicate possible seasonality in every year and six months, respectively. Small peak at frequency 1/3 indicates possible cyclicity in every 36 months.

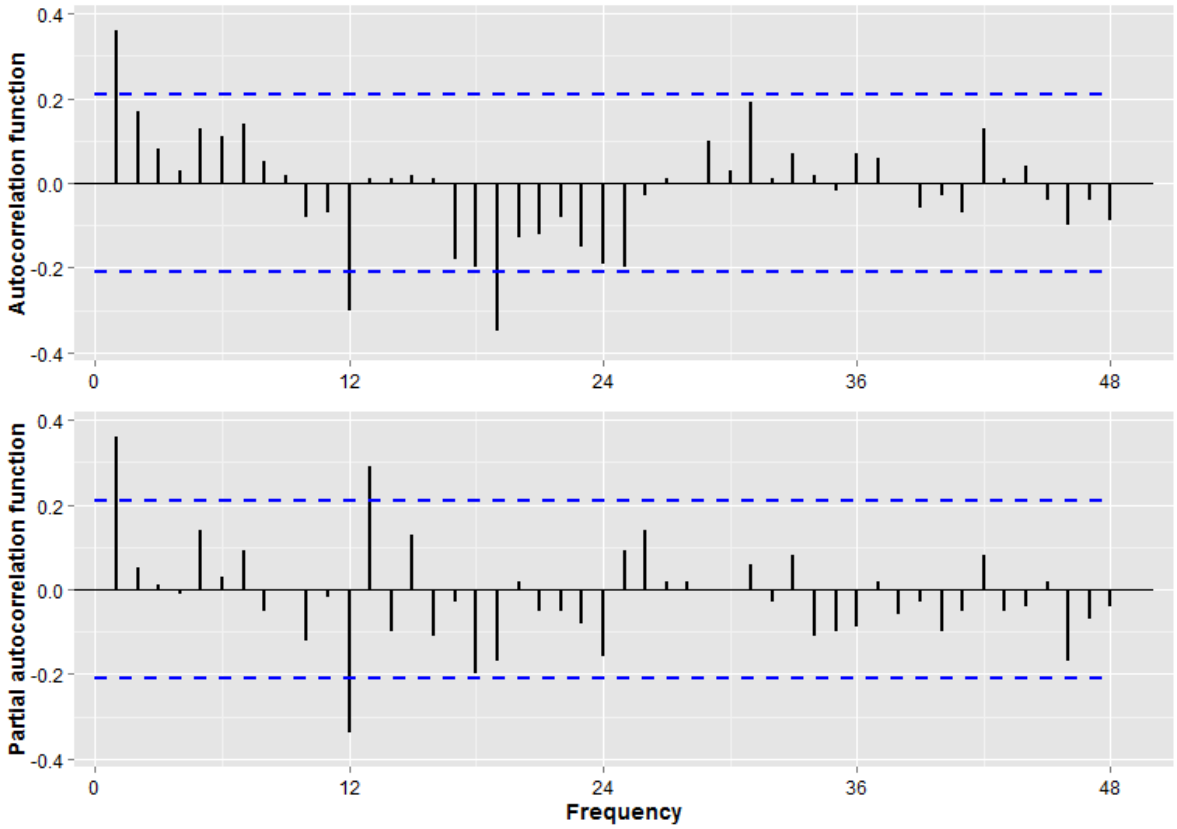

Figure 4. 4. Autocorrelation function (Top) and partial autocorrelation function (Bottom) of 12th order differenced national enlarged mesenteric lymph node prevalence (polynomial regression adjusted). Frequency is month, indicating significant correlations for every 12 months i.e. once a year.

Table 4. 2. Results of the second-order polynomial regression and multiplicative seasonal autoregressive integrated moving average model.

	Second order polynomial regression			**SARIMA**	
	Month (1st)	**Month (2nd)**	**Intercept**	**AR**	**SMA**
Coefficient	-0.04	-0.03	1.07	0.45	-1.00
S.E.	0.01	0.01	0.05	0.10	0.32
P value	< 0.01	< 0.001		< 0.01	< 0.001

Key : S.E., standard error of the coefficient; AR, autoregressive; SMA, seasonal moving average; 1st, first order; 2nd, second order

4.3.3. LEP Scoring system

- **Baselines**

The baselines for comparison of longitudinal EML prevalence for each farm are illustrated in Figure 4. 2 for the linear model (Top) and the SARIMA model (Bottom). Standard errors of the linear and the SARIMA model were 0.17 and 0.19, respectively, indicating the residual of the SARIMA model being slightly more dispersed.

- **LEP Score**

Among 1,017 commercial farms, 465 farms (45.7 %) showed no evidence of having higher EML prevalence than the baseline estimated by linear regression, suggesting those farms were categorised as having a low level of MAP infection. The median LEP score among the commercial farms among the remaining 552 commercial farms was 0.13, with an interquartile range from 0.06 to 0.24. Among them, 138 (13.6 %) farms were categorised as being at high level of MAP infection in the scoring system using the linear regression baseline.

In comparison for the scoring system using the SARIMA model-driven baseline, 478 farms (47.0 %) were categorised as low level of MAP infection. For the other 539 commercial farms, median LEP score was 0.12 (Interquartile range: 0.06 ~ 0.22). The number of farms categorised as high level of MAP infection in the SARIMA baseline scoring system was 134 (13.2 %). Figure 4. 5 and Table 4. 3 show the number of farms and LEP scores of each strata of MAP infection level. Figure 4. 6 demonstrates examples of using the EML prevalence score for one commercial farms in each of the three categories (low, moderate, high) of MAP infection.

The observed agreement between two scoring systems using the baselines from the linear regression and SARIMA model was 0.97, while the expected agreement was 0.40, resulting in the kappa statistics of 0.96 with the P value of < 0.001.

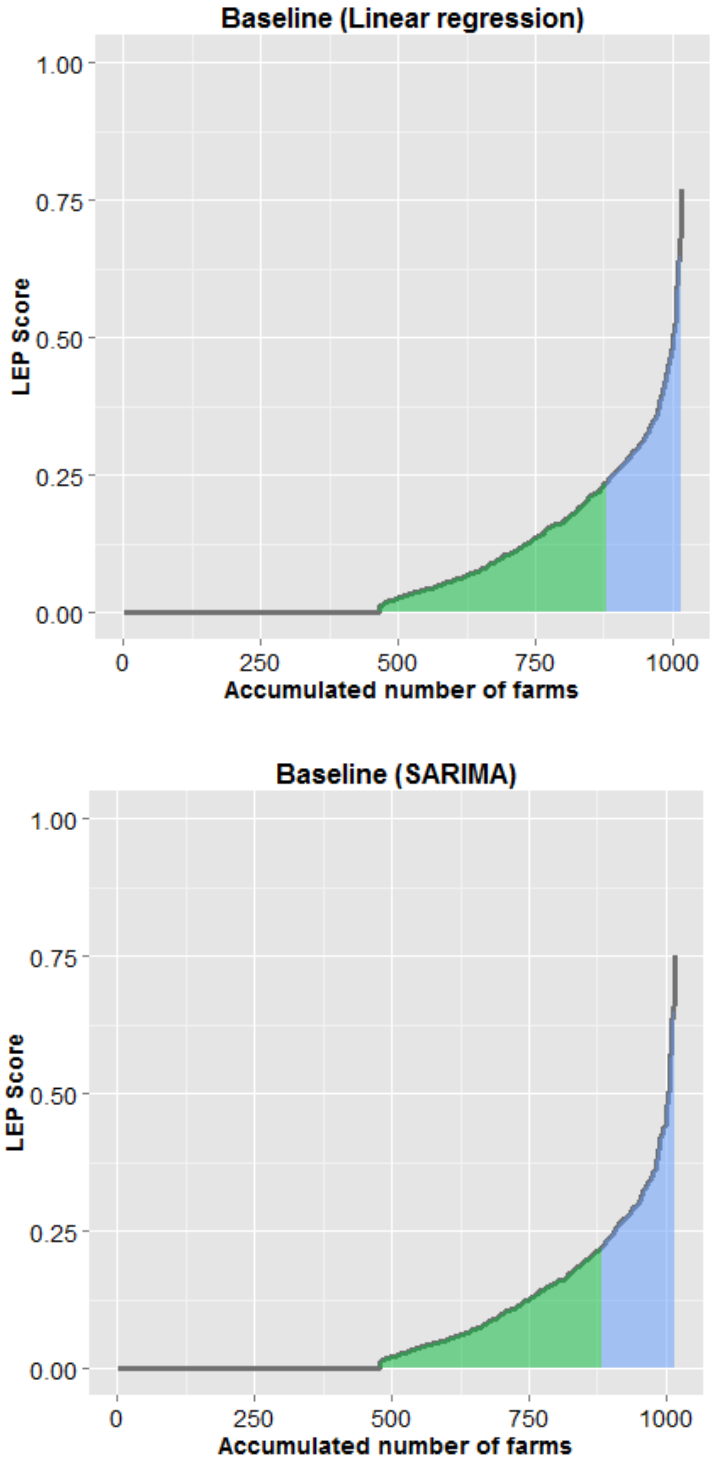

Figure 4. 5. Distribution of LEP scores of each stratum of MAP infection level using linear regression (Top) and SARIMA (Bottom) driven baselines. X-axis indicates the accumulated number of commercial farms. Line at zero of LEP score indicates farms in low level of MAP infection. Green and blue area indicate moderate and high level of MAP infection, respectively.

Table 4. 3. The number of farms and LEP scores of each stratum of MAP infection level. The MAP infection level is based on the comparisons with the baselines from linear regression and multiplicative seasonal autoregressive integrated moving average (SARIMA) models.

MAP infection	Linear Baseline			SARIMA Baseline		
	Cut-off of LEP score	Number of farms	Median LEP score (IQR)	Cut-off of LEP score	Number of farms	Median LEP score (IQR)
Low	0	465 (45.7 %)	-	0	478 (47.0 %)	-
Moderate	0.01 ≦ < 0.24	414 (40.7 %)	0.09 (0.05, 0.16)	0.01 ≦ 0.22 <	405 (39.8 %)	0.09 (0.05, 0.15)
High	≧ 0.24	138 (13.6 %)	0.32 (0.27, 0.41)	≧ 0.22	134 (13.2 %)	0.30 (0.27, 0.38)

Key : LEP, longitudinal enlarged lymph node prevalence; IQR, interquartile range; MAP, *Mycobacterium avium* subspecies *paratuberculosis*.

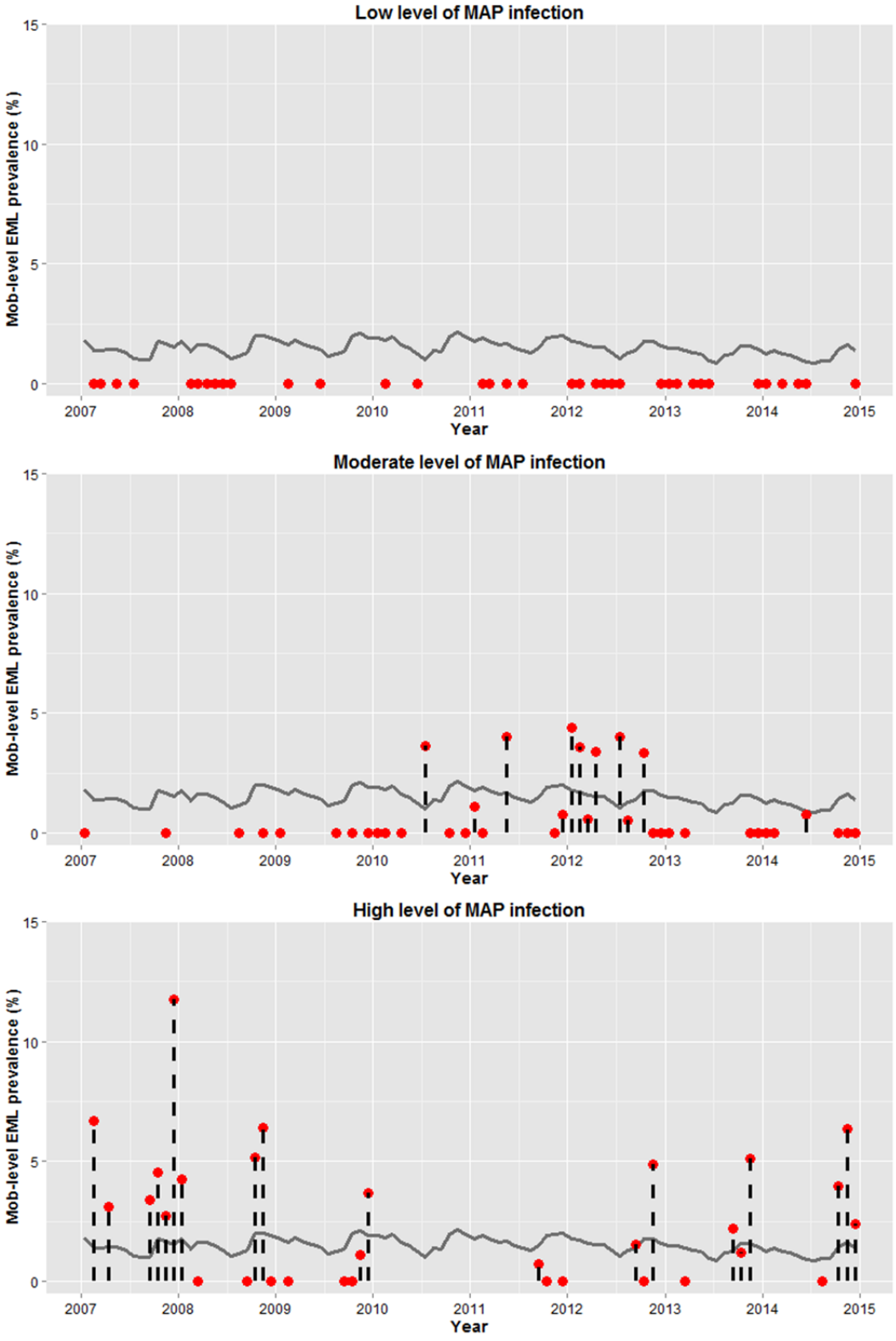

Figure 4. 6. Example deer commercial farms in each category of MAP infection level. Grey line is the baseline estimated by multiplicative seasonal autoregressive integrated moving average model, red dot is the mob submission, and black dashed line is the mob-level enlarged mesenteric lymph node prevalence in each submission.

4.4. Discussion

As the described Johne's disease surveillance system is an example of syndromic surveillance, the EML scoring system is naturally associated with diagnostic uncertainty which may also be described as statistical 'noise' (Robertson, Nelson, Ying C. MacNab, & Lawson, 2010). It indicates that the system may be limited and, consequently, that notification letters to suppliers may not provide accurate information to farmers due to false positive scoring. With the aim of using the available binary EML signals for the development of a robust farm-level Johne's disease monitoring system, we used a novel approach for removing some of the statistical noise inherent in the JML data.

EML prevalence that analysed in this study may be under-reported by meat inspectors. According to Glossop et al. (Glossop et al., 2007), the sensitivity of meat inspectors for the detection of EML was 68.0% using photographs of normal and abnormal mesenteric lymph nodes. However, when sensitivity was again examined by parallel scoring by experienced investigators in selected DSPs, the sensitivity was only 13.3 % (Jamie C. Hunnam, Wilson, Heuer, Stringer, & Mackintosh, 2013). The research team explained that the first study established the meat inspectors' capability of detecting EML, whereas the second study addressed the actual real-life detection rate. These studies emphasised that Johne's surveillance data will have to be interpreted with great caution for estimating both farm and national level prevalence of MAP infection, and even more so Johne's disease in deer. Nevertheless, a crucial assumption in syndromic surveillance is that the data reflect the change in the level of disease over time, not the exact prevalence per se. Thus, the JML programme is an example for syndromic surveillance, as data acquired for the surveillance are not presumed to reflect the true and total burden of disease in the population (Dórea et al., 2011). Also, under-reporting can be treated as a random error as it is not limited within the specific period of time. We therefore concluded that Johne's disease surveillance using the JML system is likely to be a useful tool for monitoring temporal trends of the disease in the population, but that it may be limited with regard to the disease on individual farms. As pointed out earlier, latter aspect will have to be subjected to validation by comparison of farm-level EML scores with Johne's disease incidence on the same farm.

The study population of this study was restricted to the mob sizes over 20 deer and to commercial farms. This was an arbitrary decision to reduce variation in the mob-level prevalence of EML. Consequently, approximately 25 % of commercial farm supplying deer to DSPs during the study period were excluded. The benefit, however, was that statistical noise was substantially reduced from the raw EML prevalence of the remaining farms, resulting in more precise estimation of MAP infection on farm. Moreover, in order to reduce the noise, only young deer were included in the analysis. Studies have suggested that mature deer are those surviving MAP infection, hence may be more resistant against clinical progression to Johne's disease than young deer (Jaimie C. Hunnam et al., 2009; Stringer et al., 2013). This would explain why the EML prevalence is lower in mature than young deer. Although mature deer could be infected with MAP in early life and remained at subclinical stage, we believe that discarding inspection data from old deer increased the power of the scoring system as an identifier of farms in high level of MAP infection and hence the possible extent of Johne's disease on farm.

Unlike other research with far lower statistical power (Stringer et al., 2013), the prevalence of EML in the JML data was slightly but significantly higher in stags (1.1 % vs. 0.8 %). Though statistical method would indicate a significant difference, the difference was only 0.3 %, suggesting that, from a clinical perspective, EML prevalence of stags and hinds were similar in this study.

For describing the national EML prevalence using seasonal models, we aggregated the number of deer in monthly intervals. While aggregating the information into certain period of times captured the overall pattern of EML prevalence at entire industry level, the method did not adequately consider the uncertainty of EML prevalence due to between and within farms variation. In order to incorporate those variances, mixed effect linear regression could have been applied. That analysis would have EML prevalence in each mob as an outcome, and the same explanatory variables as in the linear model of this study, while including farm as a random effect. By doing so, the expected national EML prevalence would be described adjusting for different sources of variation caused by clustering of both carcasses in mob and mobs in farm. However, the predicted value of the random effect model then is an approximation of the non-weighted national EML prevalence. In other words, all the mob-level prevalence estimates in a certain month are treated equally, because the unit of observation in random

effect model is mob-level EML prevalence. As a result, the random effect cannot incorporate the contribution of mob size into the national EML prevalence. To overcome this issue, mixed effect logistic regression with carcass-level EML status as an outcome variable could have been used. However, the logistic model was not able to be converged because of the computational limit.

In this study, the baseline was calculated by the multiplication of the standard error with 3.09. Roughly speaking, using 3.09 indicates that estimated national EML prevalence based on the model should be under the baseline in 999 out of 1,000 times. So any value over the baseline can be assumed as an obvious sign of an unexpectedly high monthly prevalence of EML. This method reduced the statistical noise in the data. The next step was calculating a score which also adjusted for the chance effect that a mob was an outlier due to a small group size (few carcasses). These scores would be low for small, and potentially high for large mobs. An average score over all supplied mobs would indicate that a farm supplied deer with consistently high EML prevalence, and this may be associated with a high incidence of MAP infection and possibly Johne's disease on farm. However, no evidence exists to date that EML at slaughter is associated with Johne's disease on farm. Our algorithm of identifying high MAP infection farms should now be used to validate the system (e.g. Monitoring MAP infection and Johne's disease incidence on farms with high and low average LEP scores).

For the estimation of the longitudinal EML prevalence score, EML prevalence in each month from a farm was compared with the baseline. Similar techniques were used to develop control charts of production processes or syndromic health surveillance (i.e. By identifying outliers form expected time series) (Burkom & Murphy, 2007; Yahav et al., 2011). However, the expected time series are commonly based on the longitudinal pattern in the past by removing any special event as, for example, a disease outbreak. Also, those studies compared the baseline with either newly observed values or simulated values, not with each component of the past pattern itself. According to the author's knowledge, there has been no previous attempt in veterinary surveillance field to identify an outlier by comparing overall pattern with the pattern of each component. Our scoring system to identify farms at high level of MAP infection was based on the binomially distributed probability of EML prevalence of a mob being above the baseline, and then summarizing these probabilities accumulating over time for every farm. By

applying this novel approach, it was able to incorporate both the frequency and the magnitude of EML prevalence in repeated mobs from a farm contributed to the probability of the farm exceeding the baseline EML prevalence. We therefore believe that this study provided a novel approach to detecting consistent source of outliers in longitudinal surveillance data using learned patterns from past records.

Based on the LEP score of each farm, we concluded that there were more than 130 commercial farms showed high level of MAP infection in longitudinal aspect. The longitudinal pattern of EML in each farm does not necessarily represent the longitudinal prevalence of Johne's disease itself. However, if the pattern of EML would indeed reflect the level of MAP infection and further to the Johne's disease on farm, describing the level of EML beyond the estimated national average with alpha level of 0.001, would be a safe indication that the level of Johne's disease in those farms was exceptionally high. Given the latter was true, such farms could then be labelled as "super-shedders" for the propagation of infection between herds. Having high LEP score also means that they would be consistent, possibly long term sources of MAP infection and Johne's disease in the population.

The two different baselines used for the scoring system did not differ significantly. On the contrary, there was a high level of agreement between them (Cohen's kappa: 0.96, P value < 0.001), hence their merit for scoring EML was similar. One advantage of using linear regression was that the model is less complex than the SARIMA model. Therefore, linear regression model may the preferred tool to analyse time series for a syndromic surveillance system, such as JML, especially when a simple system for frequent automated outputs is required. However, using linear regression for the time series may violate the assumption of independence between mobs, indicating that variances might be underestimated. Also, in this study, the scoring system using the SARIMA baseline extracted higher number of low level MAP infection farms (465 vs. 478), hence a lower number of farms with high level of MAP infection (138 vs. 134), suggesting that the SARIMA model might be more conservative to identify farms with a "high" level of MAP infection or Johne's disease.

4.5. Conclusion

This study describes the longitudinal EML prevalence of commercial farmed deer in New Zealand. Overall, the national carcass-level EML prevalence fluctuated around 1 % with strong seasonal variation between 2007 and 2014. We suggest a novel method to identify farms with a consistently high EML prevalence by comparing the longitudinal pattern of data from each source with the baseline and adjusting for uncertainty due to small mob size. Linear regression and SARIMA models were used for estimating the baseline, both with similar quality of detecting farms at risk for MAP infection. This system may be applicable to different types of surveillance or monitoring data. This study found that about 13 % of commercial deer farms had a high level of EML prevalence, hence probably a relatively high level of MAP infection. However, further research is required to compare farms with a high EML prevalence with the on-farm incidence of Johne's disease.

CHAPTER 5

Space-time clustering of Johne's disease related mesenteric lymph node pathology in commercial deer farm in New Zealand from 2007 to 2014

5.1. Introduction

Johne's disease (JD), caused by infection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is an incurable, chronic granulomatous enteritis inducing diarrhoea, weight loss and death of cloven hoofed animal species (Collins, 2003). As MAP can evade the immune response of the host, it proliferates in lymph nodes after absorption and causes enlargement of mesenteric lymph node with or without necrosis, which is the predominant histopathological symptom in deer (Jamie C. Hunnam, 2011). Since the first confirmation of a case in 1979, New Zealand has been one of the endemic countries with JD infection in farmed deer (Geoffrey W. de Lisle et al., 2005; Geoffrey W. de Lisle et al., 1993; Stringer et al., 2013; Verdugo et al., 2014). Thus, for the purpose of decreasing the burden of the disease of the industry, Johne's Management Limited (JML) was established in late of 2006. One of the rolls of JML is conducting the surveillance on the trend of Johne's disease in deer using mesenteric lymph node at slaughter. By monitoring the disease and notifying farmers, the JML programme motivates producers to initiate disease control for reducing the prevalence of the disease.

In chapter 4, we presented a methodology for identification of commercial deer farms with consistently high prevalence of enlarged mesenteric lymph nodes (EML) of young deer at slaughter, which may indicate that there is a relatively high rate of MAP infection in the slaughter mob (Jaimie C. Hunnam et al., 2013) and possibly also a high incidence of JD on the source farm. However, no evidence is currently available for the latter. By comparing the longitudinal EML prevalence of each farm with the model-estimated baseline of national EML prevalence, we examined the temporal trend of the EML prevalence in each farm. The definition of a high farm-level MAP infection was based on the accumulated EML score exceeding the baseline over time. However, the previous chapter did not consider and adjust for spatial factor.

The analysis of space-time interactions aims to examine the tendency of events to cluster in space and time. With the aid of statistical and computational improvement in recent years, the investigation of space-time interaction of infectious disease has been widely applied in both human and veterinary medical fields (Alton, Pearl, Bateman, McNab, & Berke, 2013). One obvious advantage of investigating the space-time clustering of infectious disease is that it can cope with both spatial and temporal trends of disease transmission, reflecting the patterns of disease spread more realistically. Thus, more insight can be gained about disease occurrence and spread from considering space-time interactions when analysing disease surveillance data.

Therefore, the aims of this study were; 1) To detect space-time clusters of farm-level EML prevalence by combining time series with spatial models, and 2) To adjust time trends in EML surveillance data for potential spatial effect.

5.2. Materials and methods

5.2.1. Data

JML manages a surveillance programme of JD in deer in New Zealand, monitoring individual carcass with EML at deer slaughtering premises (DSP). During the slaughtering process, meat

inspectors investigate the size of mesenteric lymph node, and record whether its size being over 55 mm in circumference along with the age and weight of carcass, farm identifier and farm's geographical coordinates. As EML (> 55 mm) is associated with MAP infection of carcasses and was suggested to be a typical subclinical sign of JD in farmed deer, farms submitted deer with at least one EML positive deer among the mob currently receive a notification letter alerting the farmer that JD may be a potential problem. However, EML is not pathognomonic for JD as it can be caused by other diseases such as bovine tuberculosis or yersiniosis (Jerrett et al., 1990; Robinson et al., 1989). It can therefore be a false positive signal in JML surveillance programme, which is based on non-specific "syndromes".

Therefore, the definition of a possible 'JD problem farm' needs to differentiate a 'high' EML prevalence from an expected 'prevalence noise' caused by such other factors. Hence, the analysis of the pattern of EML in deer should remove the noise in the data. The noise can be reduced by extracting observations from commercial farms with a sufficiently large mob size. In this study, JML data from 2007 to 2014 were used. An observation was discarded if it had missing values or was a duplicate. A commercial farm was defined as having submitted deer to DSP at least three consecutive years until 2014. Among the observations from commercial farms, we decided to use the data of only young deer with the aim of decreasing the false positives because EML due to other causes is more likely to occur in adult than young deer (Jaimie C. Hunnam et al., 2009; Mackintosh et al., 2010). To increase precision, observations from mobs smaller than 20 deer were excluded. Mob size was defined as the total number of deer submitted by a farm to DSP in one month. Farms were categorised as having either low, moderate, or high level of MAP infection, based on the longitudinal frequency and magnitude of exceeding the expected baseline of national EML prevalence. Details of the methodology are described in the previous chapter. Farms without geo-spatial location information were removed from space-time analysis.

5.2.2. Statistical analysis

Kulldorff's space-time scan statistic was used to detect and locate the significant space-time clusters. Space-time scan statistic is performed by describing a cluster of event as a cylinder in three

dimensions, with the base and height representing the space and time, respectively. Within a cylinder, spatial circles with varying radius are projected on the map, which can contain up to 50 % of the population at risk. The height of the cylinder reflects time and can be range from zero up to 50 % of the study period. Hypothetically, the events of interest are clustered in space and time if the observed number of events in a cylinder is larger than expected, assuming the number of events is either Poisson (count) or Bernoulli (positive/negative) distributed. The respective distribution is chosen depending on whether an observation is recorded as an aggregated figure (e.g. count of EML of a farm in a calendar month), or at individual level (e.g. positive vs. negative). The expected number of events is estimated based on the null hypothesis that the events are randomly distributed in space and time. The likelihood ratio of the probability of having cluster divided by the probability of the null hypothesis determines the significance for a given cluster among various simulated cylinders. The P value for a cluster associated with a maximum likelihood ratio can be estimated by Monte Carlo simulation. It is given by the Rank/(Iteration + 1), where Rank is the rank of the test statistics of an observed value, and Iteration is the total number of iterations in the Monte Carlo simulation.

In this study, the event of interest was the number of EML positive young deer in each mob, where the mob was defined as an aggregated number of deer in monthly intervals for each farm. To reduce statistical noise and thereby the false positive rate, the number of EML positive in each mob was adjusted for the expected model-estimated baseline number. The expected model-estimated baseline number was calculated by the product of each mob size and EML baseline prevalence defined by time series analysis. The number of EML positive deer in each mob was estimated by subtraction of observed EML positives by the estimated baseline number. The adjusted number of EML deer in each mob was assumed to follow a Poisson distribution. Illustration of the EML data projected for the space and time scan statistic is shown in Figure 5. 1. As there were no preceding studies describing the spatial cluster of Johne's disease in farmed deer, we arbitrarily restricted the maximum radius to 100 km while containing no more than 50 % of the population at risk. Similarly, a maximum temporal cluster size of 50 % of the study period was used. However, the minimum temporal cluster was set as 12 months. The number of replications for the Monte Carlo simulation was set to 999, indicating the lowest achievable

P value was 0.001 (i.e. 1/(999 + 1)) for the highest possible rank. A significant space-time cluster was defined as the cluster with P value less than 0.05 in this study. For secondary clusters, only clusters that did not overlap geographically with a primary cluster were reported in order to describe the size and location of clusters (Abatih, Ersbøll, Wong, & Emborg, 2009).

After a list of farms within significant space-time cluster generated by space-time scan statistic above was retrieved, the number of farms were inspected in respect to longitudinal level of MAP infection of each farm. Data were manipulate in the STATA (StataCorp, 2013), and the space-time scan statistic test was carried out with the SaTScan™ v9.4.1 (Kulldorff, 2015). The result of the space-time scan statistic was read back into the R (R Core Team, 2014) for reporting and plotting of map.

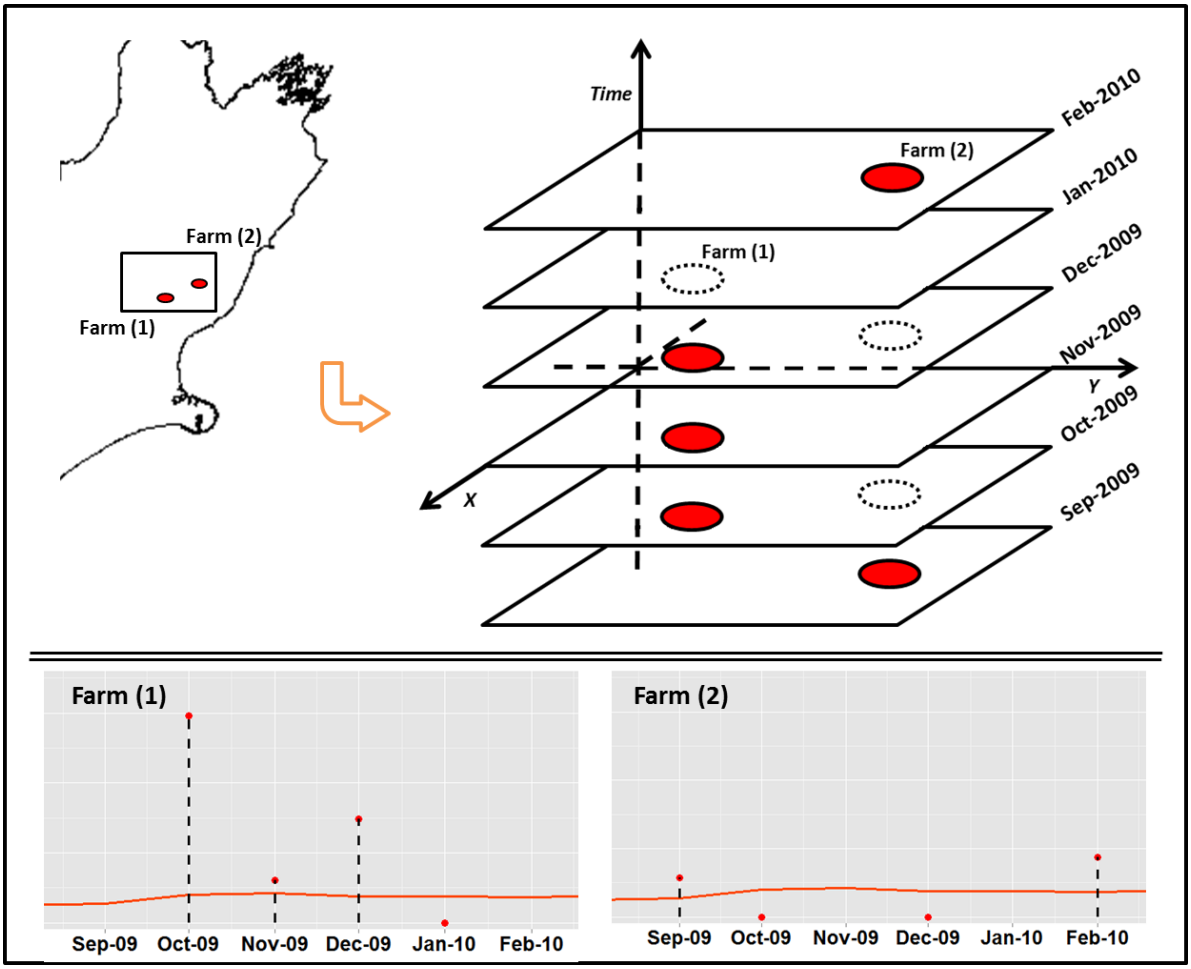

Figure 5. 1. Conceptual illustration of EML surveillance data being projected for the space-time scan statistic. Orange solid lines (Bottom graphs) indicate the number of expected enlarged mesenteric lymph node (EML) positive deer based on the model. Red dots are submission of mob in each month, and dashed vertical lines are the number of EML positive deer in each mob. The number of EML positive deer adjusted for the baseline in each submission is projected in 3-dimensional space (Top right figure). Dotted circles indicate the absence of an unusually high number of EML positive deer due to the adjustment for the baseline.

5.3. Results

5.3.1. Study population

Observations of young deer from commercial farm with mob size larger than 20 deer was extracted from the JML dataset for the analysis, resulting in 2,092,451 deer from 1,017 farms. Among those farms, only 949 farms had geo-spatial coordinates, resulting in observations of 1,901,931 deer slaughtered from 22,506 mobs were analysed for the space-time scan statistic. 447 farms showed no evidence of EML over the baseline, resulting in categorised into low level of MAP infection. Among the farms with the score above the zero, 125 farms were categorised in high level of MAP infection, consisting 29.5 % of deer in the study population. Remained 377 farms were categorised into moderate level. The number of deer with EML positive was 7,036 and average population was 19,791 deer per year during the study period, resulting in 4,443.9 annual cases per 100,000 deer. Among deer with EML positive, 5,684 deer (80.8 %) were from the farms in high level of MAP infection.

5.3.2. Spatial distribution of commercial farms

Figure 5. 2 illustrates the spatial distribution of commercial farms in New Zealand between 2007 and 2014. Among 949 commercial farms, 275 farms (29.0 %) were located in North Island. Figure 5. 2 also describes the location of farms with different degree of longitudinal MAP infection. For the 447 and 377 farms in low and moderate levels, 51.0 % and 12.5 % were located in the North Island, respectively. All the farms that having high level of MAP infection were distributed in the South Island.

5.3.3. Temporal distribution of high EML

Noise embedded in the number of EML positive deer per each mob was reduced by subtracting expected number of deer with EML, based on the time series analysis (i.e. Polynomial regression + multiplicative seasonal autoregressive integrated moving average). Noise adjusted number of EML

positive deer in the study population is illustrated in Figure 5. 3 as time series. The overall number showed distinctive seasonal fluctuation with the peak in summer.

5.3.4. Space-time clustering

According to the space-time scan statistic, 21 space-time clusters were detected. Among them, 17 clusters were statistically significant (P value < 0.05). All those clusters were located in the South Island, and there was no space-time cluster in the North Island. The largest and smallest radii of clusters were 97.8 km and 0 km, respectively. Radius of 0 km indicates that, up to adjacent 100 km, the most likely cluster was the one only including the farm itself. The description of significant clusters is presented in Table 5. 1, and illustration of six clusters having larger radii than 0 km is described in Figure 5. 4 along with the location of commercial farms.

5.3.5. Level of MAP infection and Space-Time clustering

Among 949 commercial farms, 144 farms were included in any of significant space-time cluster. Stratifying by the level of MAP infection, the numbers of farms at low and moderate levels that included in the clusters were 29 (6.5 %) and 57 (17.8 %), respectively. For farms at high level of MAP infection, 58 farms (46.8 %) were included in space-time clusters. Table 5. 1 presents the number of farms at high level of MAP infection that included in each cluster. Among 11 farms that being detected as most likely cluster as itself, five farms were in high level, and others were in moderate level.

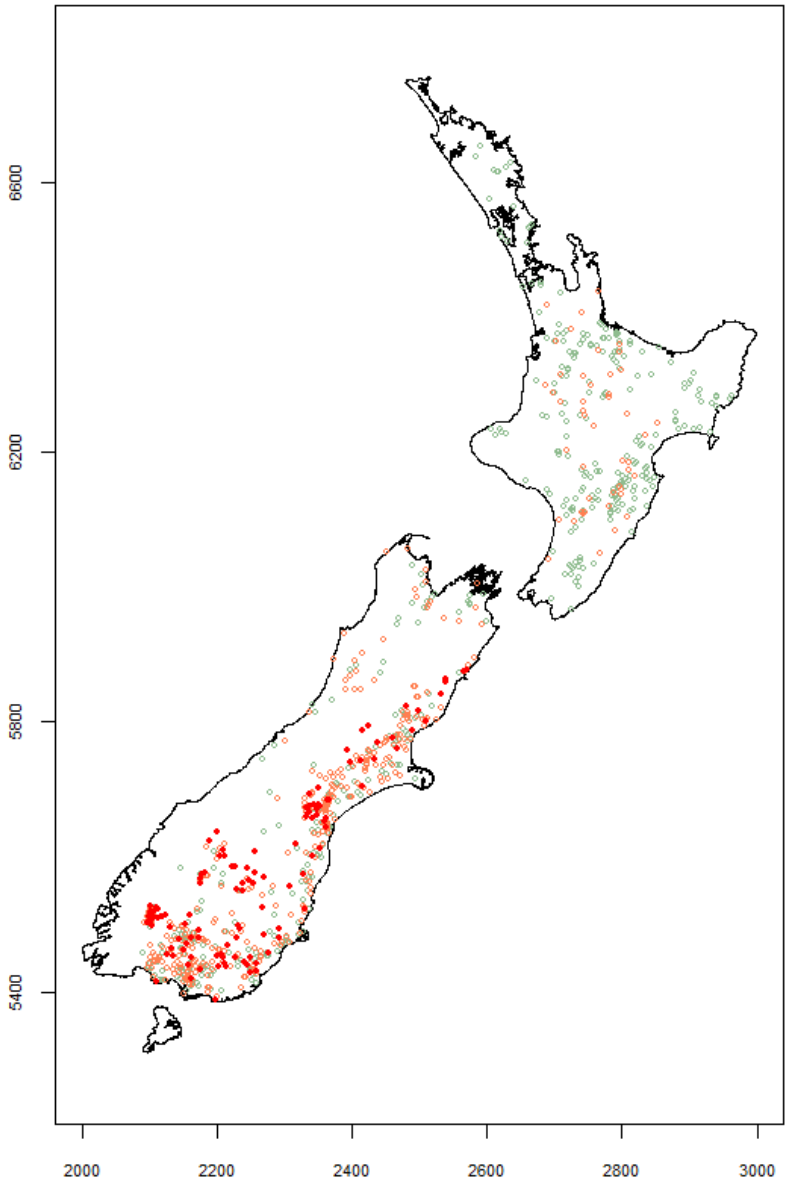

Figure 5. 2. Distribution of commercial deer farms in New Zealand. Green and orange empty circles indicate the farms with low and moderate level of longitudinal MAP infection, respectively. Red solid circle is the farms with high level of MAP infection.

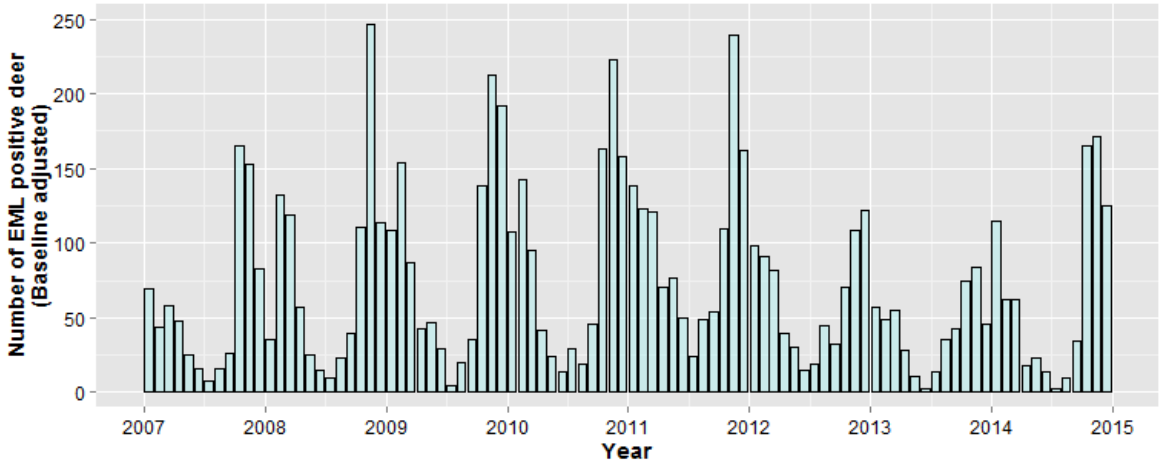

Figure 5. 3. Overall number of deer with enlarged mesenteric lymph node (EML). The overall number of deer in this graph is the baseline adjusted value, indicating that the number of EML positive deer in each mob is subtracted by the number of expected EML positive deer based on the model.

Table 5. 1. Result of space-time scan statistic of adjusted number of EML positives in commercial deer farm from 2007 to 2014. Only the statistically significant clusters are presented.

Cluster ID	Cluster Date	Duration in month	# Farms	# Farms in high level	Observed cases	Expected cases	Relative risk	P value
A	Feb. 2008	47	96	38	2028	597.5	4.4	0.001
B	Nov. 2008	48	20	8	232	85.2	2.8	0.001
C	Oct. 2007	42	6	3	395	68.3	6.1	0.001
D	Nov. 2008	13	3	0	12	1.1	10.5	0.003
E	Oct. 2008	27	5	2	78	12.2	6.4	0.001
F	Jan. 2014	12	3	2	45	2.8	16.1	0.001
G	Jan. 2014	12	1	1	78	10.2	7.8	0.001
H	Nov. 2008	40	1	1	60	1.7	36.8	0.001
I	Nov. 2011	12	1	1	12	0.3	45.5	0.001
J	Sep. 2010	12	1	0	15	2.3	6.5	0.018
K	Jan. 2007	12	1	0	26	7.4	3.6	0.04
L	May 2008	12	1	0	22	1.3	17.2	0.001
M	Nov. 2008	37	1	1	78	8.0	9.8	0.001
N	Dec. 2011	12	1	0	19	1.2	15.6	0.001
O	Oct. 2008	12	1	0	12	0.4	33.9	0.001
P	Feb. 2012	32	1	1	14	1.9	7.6	0.007
Q	Sep. 2008	38	1	0	24	2.8	8.6	0.001

Key: ID, identifier; #, Number of; high level, high level of MAP infection.

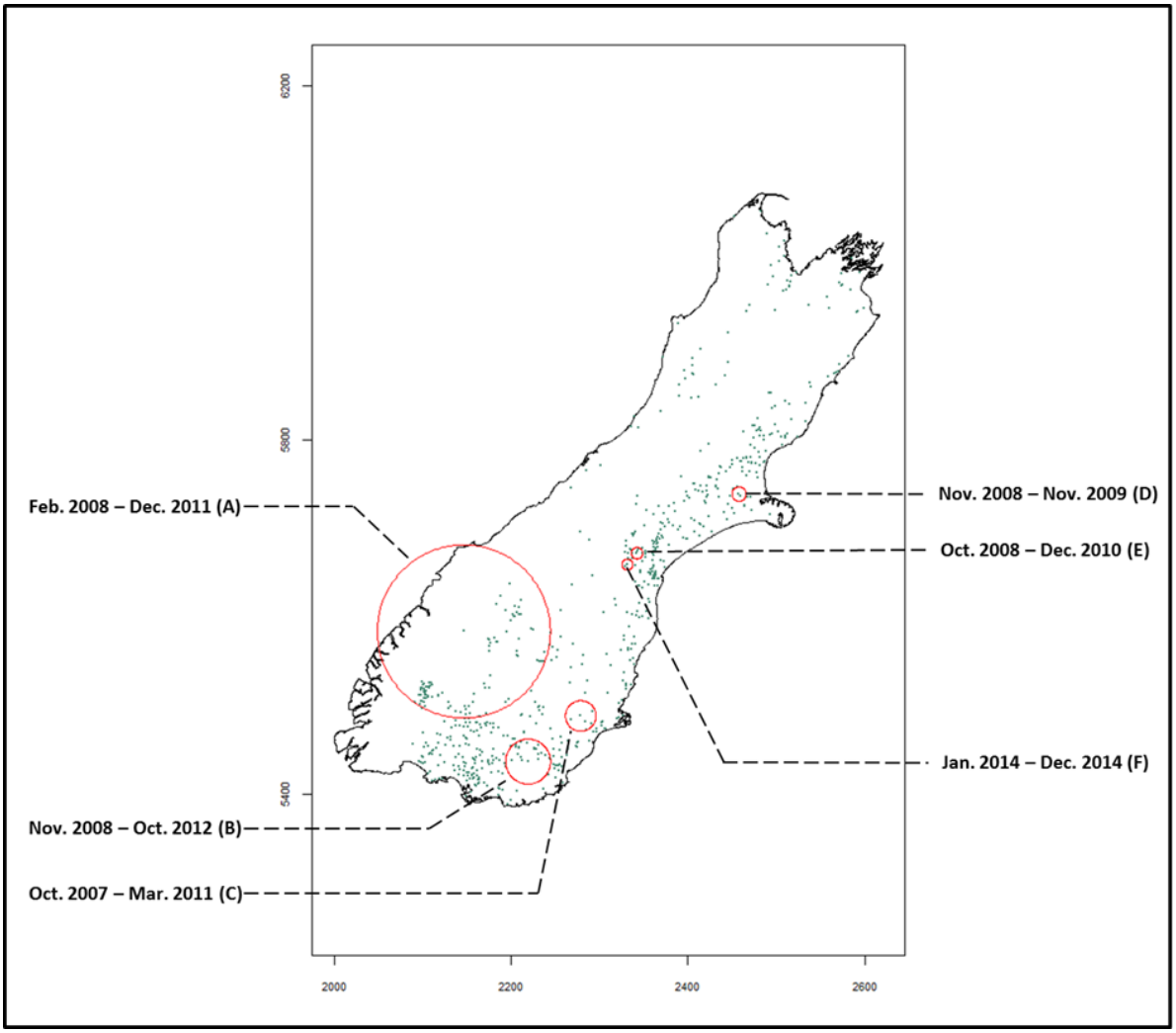

Figure 5. 4. Description of the significant space-time clusters of adjusted number of EML positives in commercial deer farm between 2007 and 2014. Red circles indicate the significant clusters, and green dots are commercial deer farms. Farms that being detected as most likely cluster as itself are not presented on this figure for confidentiality.

5.4. Discussion

We conducted this study to detect any evidence of cluster in EML occurrence in both space and time perspective. The number of EML positive deer in each mob was inspected to identify the location and duration of significant space-time clusters. Even though EML is not a definitive symptom indicating the infection of MAP, we used EML as a proxy for MAP infection because the proportion of MAP culture-positve mesenteric lymph node among EML classified by meat workers was 92.2% (Jaimie C. Hunnam et al., 2013). Count of EML were known to have low sensitivity due to being inaccurately classified by abattoir workers (Jamie C. Hunnam et al., 2013). However, the probability of the error was not limited to any specific period of time or location, suggesting that this error was biased the results equally over time and space. While not all farms with a high MAP infection might have detected, we believe that farms detected at those time points were those with a relatively high EML prevalence, hence a likely high MAP infection rate and a possible JD problem. A validation of the JML system can therefore use out detection methodology for comparing farms with low, moderate, or high level of MAP infection with actual on-farm observations of JD incidence.

For this study, study population of 1,901,931 slaughtered deer from 949 farms was used, constituting 53.6 % of the original study population after exclusion of the JML data with missing values or duplicated observations. Among 1,017 commercial farms, 949 farms had geographical information in data, indicating more than 90 % of commercial deer farms in New Zealand could be traced for follow-up or intervention.

More than two third of commercial farms were located in the South Island, of which 67.5 % had either a moderate or high level of MAP infection during 2007 - 2014. In the North Island, only 17.1 % of commercial farms had moderate level of MAP infection. Interestingly, none of the commercial farms in the North Island showed evidence of a high level of infection during this study period, suggesting that the North Island has a far lower prevalence of MAP infection and possibly JD. This finding agrees with several recent studies (Jaimie C. Hunnam et al., 2013; Stringer et al., 2013; Verdugo et al., 2014).

The apparent seasonal fluctuation of EML prevalence might be biased by the strong reproductive seasonality and the associated seasonal fluctuation in age of young deer at slaughter. Deer, as all other cloven hoofed species, are more susceptible to MAP infection at young age, and the later an animal is slaughtered the longer the time at risk for infection. However, other than cattle, death due to JD occurs earlier in life, hence adult deer are less likely to be clinically affected by JD as are young deer (Mackintosh et al., 2010). Therefore, the EML prevalence of adult deer was excluded from this analysis.

To test the space-time scan statistic, we restrict the radius of spatial cluster being less than 100 km with the length of temporal cluster at least 12 months. While this criterion for defining a cluster was arbitrary, we assumed that radius over 100 km was relatively large considering the geographical distribution of deer farms in New Zealand. The 12-months minimum duration of a temporal cluster was felt to be required for sufficient sensitivity for detecting EML clusters, and not too long for missing any true cluster due to a too large denominator. As those criteria were chosen before the analysis was conducted, this study may not be free from 'pre-selection bias' (i.e. Detected clusters were based on the selection criteria rather than on the underlying reality) (Pfeiffer et al., 2008).

We defined 17 statistically significant space-time clusters based on the Monte Carlo simulation. Nine of them were detected since 2008, 10 remained significantly until 2011/2012, and two were new clusters detected in the most recent period (2014). Assuming that a farm with a high MAP infection has a high JD incidence, this might indicate that Johne's disease had peaked in the past, and that the level of JD has decreased since 2012. If this was true, the findings may indicate that JD control efforts of JML started to show positive effects since late 2006 as JML is motivating producers to reduce the burden of Johne's disease in deer industry in New Zealand.

Interestingly, all the significant clusters were located in the South Island, even though a few farms were detected with a moderate level of MAP infection in the North Island. This may raise the question whether deer farms in the North Island contribute significantly less to the transmission of Johne's disease in the country and may therefore be more preferred for the replacement of breeding stock or establishment of new deer farms to the South Island. It was noteworthy that more than half of the farms

with a high level of MAP infection were not included in any of the significant space-time clusters, suggesting they might have experienced sporadic outbreaks of Johne's disease.

Even though we detected several significant clusters in space and time, they were likely not the only true space-time clusters of Johne's disease in New Zealand. This is because the analysis only included farms sending deer to a DSP for slaughter. So even if a farm had Johne's disease, this detection system would not capture it unless the farm appears in the JML data. For the farms that were included in the JML database, we believe that the number of detected space-time clusters was probably an underestimate of true clusters occurring in the population due to excluding non-commercial and small commercial farms, and due to the fairly wide buffer above the estimated baseline of EML prevalence. So a limitation may be the lack of sensitivity of detecting MAP infection herds rather than labelling non-infected farms false positive.

5.5. Conclusion

This study detected statistically significant space-time clusters of commercial deer farms with a high EML prevalence suggesting a high level of MAP infection by analysing abattoir detection of EML positive deer carcasses. However, a validation of EML at slaughter is required before any inferences about JD in the population. We suggest network analysis of MAP-infected and JD-affected farms to evaluate JD transmission and control of deer farms in New Zealand more accurately.

Bibliography

Abatih, E. N., Ersbøll, A. K., Wong, D. M. A. L. F., & Emborg, H.-D. (2009). Space–time clustering of ampicillin resistant Escherichia coli isolated from Danish pigs at slaughter between 1997 and 2005. *Preventive veterinary medicine, 89*(1), 90-101.

Alton, G. D., Pearl, D. L., Bateman, K. G., McNab, B., & Berke, O. (2013). Comparison of covariate adjustment methods using space-time scan statistics for food animal syndromic surveillance. *BMC veterinary research, 9*(1), 231.

Annonymous. (2005). *Agricultural Production Statistics: June 2005 - Tables (final).* Retrieved from http://www.stats.govt.nz/.

Barry, T. N., Wilson, P. R., & Kemp, P. D. (1999). A review of forage grazing systems to produce venison according to market signals. *Proceedings of the New Zealand Society of Animal Production, 59*, 140-144.

Becketti, S. (2013). Introduction to time series using Stata. *Stata Press books*.

Benschop, J., Stevenson, M. A., Dahl, J., Morris, R. S., & French, N. P. (2008). Temporal and longitudinal analysis of Danish Swine Salmonellosis Control Programme data: implications for surveillance. *Epidemiology and infection, 136*(11), 1511-1520.

Burkom, H., & Murphy, S. P. (2007). Automated time series forecasting for biosurveillance. *Statistics in Medicine, Forthcoming*, 06-035.

Carpenter, T. E., & Hird, D. W. (1986). Time series analysis of mycobacteriosis in California slaughter swine. *Preventive veterinary medicine, 3*(6), 559-572.

Cohen, J. (1960). A coefficient of agreement for nominal scales. *Educational and psychological measurement, 20*(1), 37-46.

Collins, M. T. (2003). Paratuberculosis: Review of present knowledge. *Acta Vet Scand, 44*(3-4), 217-221.

Diggle, P. (1990). *Time series: a biostatistical introduction*: Oxford University Press.

DINZ. (2015). Deer Industry New Zealand 2013 - 2014 Annual Report. http://www.deernz.org/sites/dinz/files/DINZ-AR-2013-14-WebFinal.pdf Retrieved from http://www.deernz.org/sites/dinz/files/DINZ-AR-2013-14-WebFinal.pdf

Dórea, F. C., Sanchez, J., & Revie, C. W. (2011). Veterinary syndromic surveillance: Current initiatives and potential for development. *Preventive veterinary medicine, 101*(1), 1-17.

Dupuy, C., Bronner, A., Watson, E., Wuyckhuise-Sjouke, L., Reist, M., Fouillet, A., . . . Perrin, J.-B. (2013). Inventory of veterinary syndromic surveillance initiatives in Europe (Triple-S project): Current situation and perspectives. *Preventive veterinary medicine, 111*(3), 220-229.

Gautam, R., Guptill, L. F., Wu, C. C., Potter, A., & Moore, G. E. (2010). Spatial and spatio-temporal clustering of overall and serovar-specific Leptospira microscopic agglutination test (MAT) seropositivity among dogs in the United States from 2000 through 2007. *Preventive veterinary medicine, 96*(1), 122-131.

Geoffrey W. de Lisle, Cannon, M. C., Yates, G. F., & Collins, D. M. (2005). *Abattoir surveillance of paratuberculosis in farmed deer.* Paper presented at the Proceedings of the 8th international colloquium on paratuberculosis.

Geoffrey W. de Lisle, Yates, G. F., & Collins, D. M. (1993). Paratuberculosis in farmed deer: case reports and DNA characterization of isolates of Mycobacterium paratuberculosis. *Journal of Veterinary Diagnostic Investigation, 5*(4), 567-571.

Glossop, J. C., Clark, R. G., Mackintosh, C. G., West, D. M., Heuer, C., & Wilson, P. R. (2007). Sensitivity, specificity and repeatability of gross inspection of enlarged,Johne's disease-suspect'mesenteric lymph nodes of farmed red deer (Cervus elaphus) by accredited meat assessors. *DEER BRANCH COURSE, 24*, 51.

Goodall, E. A., McLoughlin, E. M., Menzies, F. D., & Mcllroy, S. G. (1991). Time series analysis of the prevalence of Ascaris suum infections in pigs using abattoir condemnation data. *Animal Production, 53*(03), 367-372.

Gumbrell, R. C. (1986). Johne's disease in deer. *Surveillance, 13(4)*, 15-16.

Höhle, M., Paul, M., & Held, L. (2009). Statistical approaches to the monitoring and surveillance of infectious diseases for veterinary public health. *Preventive veterinary medicine, 91*(1), 2-10.

Hunnam, J. C. (2011). *The epidemiology of Johne's disease in New Zealand farmed deer, including validation of abattoir-based surveillance.* (Ph.D in Veterinary Clinical Science), Massey University, Palmerston North, New Zealand.

Hunnam, J. C., Heuer, C., Stevenson, M. A., & Wilson, P. R. (2009). *National slaughterhouse-based surveillance for Johne's disease in New Zealand farmed deer.* Paper presented at the Proceedings of a Deer Course for Veterinarians, Deer Branch of the New Zealand Veterinary Association.

Hunnam, J. C., Wilson, P. R., Heuer, C., Stringer, L., Clark, R. G., & Mackintosh, C. G. (2013). Association between Mycobacterium avium subspecies paratuberculosis and lymph node size in New Zealand farmed deer (Cervus elaphus). *New Zealand veterinary journal, 61*(3), 133-140.

Hunnam, J. C., Wilson, P. R., Heuer, C., Stringer, L., & Mackintosh, C. G. (2013). Sensitivity, specificity and level of agreement of meat inspector detection of abnormal lymph nodes of farmed deer (Cervus elaphus) in New Zealand. *New Zealand veterinary journal, 61*(3), 141-146.

Jerrett, I. V., Slee, K. J., & Robertson, B. I. (1990). Yersiniosis in farmed deer. *Australian veterinary journal, 67*(6), 212-214.

Jore, S., Viljugrein, H., Brun, E., Heier, B. T., Borck, B., Ethelberg, S., . . . Hansson, I. (2010). Trends in Campylobacter incidence in broilers and humans in six European countries, 1997–2007. *Preventive veterinary medicine, 93*(1), 33-41.

Kulldorff, M. (2001). Prospective time periodic geographical disease surveillance using a scan statistic. *Journal of the Royal Statistical Society: Series A (Statistics in Society), 164*(1), 61-72.

Kulldorff, M. (2015). SaTScan v9.4.1. Harvard Medical School, Boston & Information Management Services Inc., Calverton, Maryland. Retrieved from http://www.satscan.org/

Mackintosh, C. G., Clark, R. G., Thompson, B., Tolentino, B., Griffin, J. F. T., & Lisle, G. W. d. (2010). Age susceptibility of red deer (Cervus elaphus) to paratuberculosis. *Veterinary microbiology, 143*(2), 255-261.

MacPherson, L. (2014). *Agricultural Production Statistics: June 2014 (final)*.

Mandl, K. D., Overhage, J. M., Wagner, M. M., Lober, W. B., Sebastiani, P., Mostashari, F., . . . Koski, E. (2004). Implementing syndromic surveillance: a practical guide informed by the early experience. *Journal of the American Medical Informatics Association, 11*(2), 141-150.

Martínez-López, B., Perez, A. M., & Sánchez-Vizcaíno, J. M. (2011). Identifying equine premises at high risk of introduction of vector-borne diseases using geo-statistical and space-time analyses. *Preventive veterinary medicine, 100*(2), 100-108.

McIlroy, S. G., Goodall, E. A., Stewart, D. A., Taylor, S. M., & McCracken, R. M. (1990). A computerised system for the accurate forecasting of the annual prevalence of fasciolosis. *Preventive veterinary medicine, 9*(1), 27-35.

Pfeiffer, D., Robinson, T. P., Stevenson, M. A., Stevens, K. B., Rogers, D. J., & Clements, A. C. A. (2008). *Spatial analysis in epidemiology*: Oxford University Press Oxford.

Pollard, J. C. (1993). *Behavioural quantification of welfare in farmed red deer.* Paper presented at the Proceedings of the New Zealand Society of Animal Production.

R Core Team. (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org/

Robertson, C., Nelson, T. A., Ying C. MacNab, & Lawson, A. B. (2010). Review of methods for space–time disease surveillance. *Spatial and Spatio-temporal Epidemiology, 1*(2), 105-116.

Robinson, R. C., Phillips, P. H., Stevens, G., & Storm, P. A. (1989). An outbreak of Mycobacterium bovis infection in fallow deer (Dama dama). *Australian veterinary journal, 66*(7), 195-197.

Sanchez-Vazquez, M. J., Nielen, M., Gunn, G. J., & Lewis, F. I. (2012a). National monitoring of Ascaris suum related liver pathologies in English abattoirs: A time-series analysis, 2005–2010. *Veterinary parasitology, 184*(1), 83-87.

Sanchez-Vazquez, M. J., Nielen, M., Gunn, G. J., & Lewis, F. I. (2012b). Using seasonal-trend decomposition based on loess (STL) to explore temporal patterns of pneumonic lesions in finishing pigs slaughtered in England, 2005–2011. *Preventive veterinary medicine, 104*(1), 65-73.

Sargeant, J. M., Shoukri, M. M., Martin, S. W., Leslie, K. E., & Lissemore, K. D. (1998). Investigating potential risk factors for seasonal variation: an example using graphical and spectral analysis methods based on the production of milk components in dairy cattle. *Preventive veterinary medicine, 36*(3), 167-178.

Serfling, R. E. (1963). Methods for current statistical analysis of excess pneumonia-influenza deaths. *Public health reports, 78*(6), 494.

Shumway, R. H., & Stoffer, D. S. (2010). *Time series analysis and its applications: with R examples*: Springer Science & Business Media.

StataCorp. (2013). Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.

Stringer, L., Wilson, P. R., Heuer, C., Hunnam, J. C., Verdugo, C., & Mackintosh, C. G. (2013). Prevalence of Mycobacterium avium subsp. paratuberculosis in farmed red deer (Cervus elaphus) with grossly normal mesenteric lymph nodes. *New Zealand veterinary journal, 61*(3), 147-152.

The PostgreSQL Global Development Group. (2015). PostgreSQL 9.3. Retrieved from http://www.postgresql.org/

Tsui, F. C., Wagner, M. M., Dato, V., & Chang, C. C. (2001). *Value of ICD-9 coded chief complaints for detection of epidemics.* Paper presented at the Proceedings of the AMIA Symposium.

Verdugo, C., Jones, G., Johnson, W. O., Wilson, P., Stringer, L., & Heuer, C. (2014). Estimation of flock/herd-level true Mycobacterium avium subspecies paratuberculosis prevalence on sheep, beef cattle and deer farms in New Zealand using a novel Bayesian model. *Preventive veterinary medicine, 117*(3), 447-455.

Vial, F., & Reist, M. (2014). Evaluation of Swiss slaughterhouse data for integration in a syndromic surveillance system. *BMC veterinary research, 10*(1), 33.

Yahav, I., Lotze, T., & Shmueli, G. (2011). Algorithm Combination for Improved Performance in Biosurveillance *Infectious Disease Informatics and Biosurveillance* (pp. 173-189): Springer.