Epidemiological studies of Bovine Digital Dermatitis in pasture-based dairy herds in the North Taranaki, New Zealand

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Abstract

Bovine digital dermatitis (BDD) is the most important infectious cause of lameness in dairy cattle, but, until recently, has been rarely recorded in New Zealand (NZ). In 2011, the Ministry of Primary Industries summarised several case observations from 2004 to 2011, highlighting the potential for BDD to become a significant production disease on NZ dairy farms and prompting a pilot study of BDD prevalence and risk factors. However, there is currently no gold standard for the diagnosis of bovine digital dermatitis (BDD). Diagnosis of BDD was based on clinical appearance. Therefore a latent class modelling was adopted to assess the reliability of detecting BDD using visual appearance.

A survey of 224 dairy farms in the North Taranaki region of the North Island of NZ was undertaken from 09/14 to 02/15. BDD was seen in 707/60,455 cows (1.2%, 95%CI: 0.9% - 3.0%) and on 143/224 (63.8%, 95%CI: 57.5% - 70.1%) farms. The prevalence of BDD affected cows within farms ranged from 0 to 12.6%. In affected cows, 268/707 (37.9%) had a lesion in the left foot only, 263 (37.1%) in the right foot only and 177 (25%) in both feet suggesting feet-to-feet transmission within cow. To validate the uncertainty of prevalence due to inaccurate diagnosis, a Bayesian latent class model was adopted to predict the true prevalence given priors of sensitivity and specificity based on an expert opinion. However, only the posterior of the specificity 99.9% (95% probability interval (PI): 99.8% - 99.9%) was constant in each scenario of sensitivity analyses. Therefore, the true BDD prevalence could not be estimated accurately due to the uncertainty of screening sensitivity.

Risk factors were identified by a generalised linear model using information collected during the herd screening. BDD was most common in September which is likely to be related to stage of lactation and climate (wet in spring, dry in summer). More BDD cases were recorded in rotary sheds, probably because in such sheds feet could be examined for a longer period of time. Introducing cattle was shown to be a significant risk factor for having

BDD on a farm. This may suggest that BDD could be transmitted by cow-to-cow in the study area. Another significant risk factor was hoof trimming by external staff. This is likely to be related to insufficient disinfection of equipment between farms.

Whereas the proportion of affected farms appeared to be relatively high in Taranaki, the cow level prevalence was lower than reported overseas. However, comparative data are missing for New Zealand. The observed BDD prevalence was likely underestimated because small lesions could easily be missed, particularly if they were deep inter-digital, on the anterior side of the hind feet or in the front feet. Additionally, inconclusive lesions were not counted as cases in the analysis even though they were thought highly likely to be due to BDD when pictures were later shown to overseas experts. Our investigations showed that digital dermatitis is present on most farms in the North Taranaki and that this could potentially become a significant cause of lameness on dairy farms in New Zealand.

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Bovine digital dermatitis (BDD), also known as Mortellaro's disease, papillomatous digital dermatitis (PDD), hairy warts and strawberry warts, is a highly infectious disease which is often associated with lameness (R. A. Laven, 2003). BDD was described for the first time in Italy in 1974 (Cheli & Mortellaro, 1974). It was then mainly observed in intensive dairy systems and appeared to spread through these systems in the Northern hemisphere (Evans et al., 2011). BDD was described as a painful disease with substantial negative impact on milk production, body condition score, reproductive performance and animal welfare in dairy cattle (Hernandez, Shearer, & Webb, 2001, 2002; Kocak & Ekiz, 2006; Winckler & Willen, 2001).

Although the disease was reported in many countries, it appeared to be non-existent or unobserved in pasture based dairy herds of New Zealand until 2004 (Vermunt & Hill, 2004). In 2004, a three years old Holstein-Friesian bull from a 500-cow dairy farm in the North Island was considered to be affected by BDD because a foot lesion had histopathological similarity to those reported overseas. Two years later, another BDD case was sighted in the South Island and reported to the Ministry of Primary Industry (Varney & Gibson, 2006). After five years without further observations, five cases were reported in 2011. A histopathological assessment was conducted by Dr Deryck Read, an overseas pathologist with extensive BDD experience. Findings are summarised in Table 1.1.

Table 1. 1 Summary of five suspicious Bovine Digital Dermatitis Cases in New Zealand.

Case	Region	Animal			Lameness	Histological Criteria			Treponema IHC	Papilloma Virus
		Affected	Total	System		Loss of stratum Corneum	Spirochaete Mat	Dermal Inflammatory Response		
1	Waikato	1	300	Dairy	No	√	√	√	Positive in focal areas	Negative
2	Waikato	1	150	Dairy	No	√	√	√	Positive in focal areas	Negative
3	Bay of Plenty	7	42	Beef	Yes	X	√	√	Not done	Negative
4	Canterbury	1	600	Dairy	No	X	X	√	Not done	Negative
5	Waikato	1	167	Dairy	No	X	X	√	Negative	Negative

Source: Review of Recent Bovine Digital Dermatitis-Like Lesions in Cattle (Andel, Rawdon, Thompson, & Vink, 2012)

Few of the BDD cases observed in New Zealand was associated with lameness. However, it was suggested that BDD may have a potential to be contagious and cause lameness in cattle while remaining clinically inapparent. A typical BDD lesion can be seen on the skin of the rear inter-digital cleft, midline just above the bulb of the heel, further down between the digits, or even as far up as in the area of the coronary band (Holzhauer, Bartels, Dopfer, & van Schaik, 2008; Parkinson TJ, Vermunt JJ, & Malmo J, 2012). According to a New Zealand description (personal communication with Neil Chesterton), a big wart-like lesion can be found in the affected area or as small, dry scab when it is in healing stage (Figure 1.1). On palpation, the skin presents with a rough surface. There is no swelling of the claw. The lesion is usually overlooked unless feet and claws are thoroughly cleaned which can best be done while standing in the milking shed. BDD lesions were the most commonly found in rear feet rather than front feet (Cramer, Lissemore, Guard, Leslie, & Kelton, 2008). Between rear feet, the prevalence of BDD did not present a significant difference (Holzhauer, Hardenberg, Bartels, & Frankena, 2006).

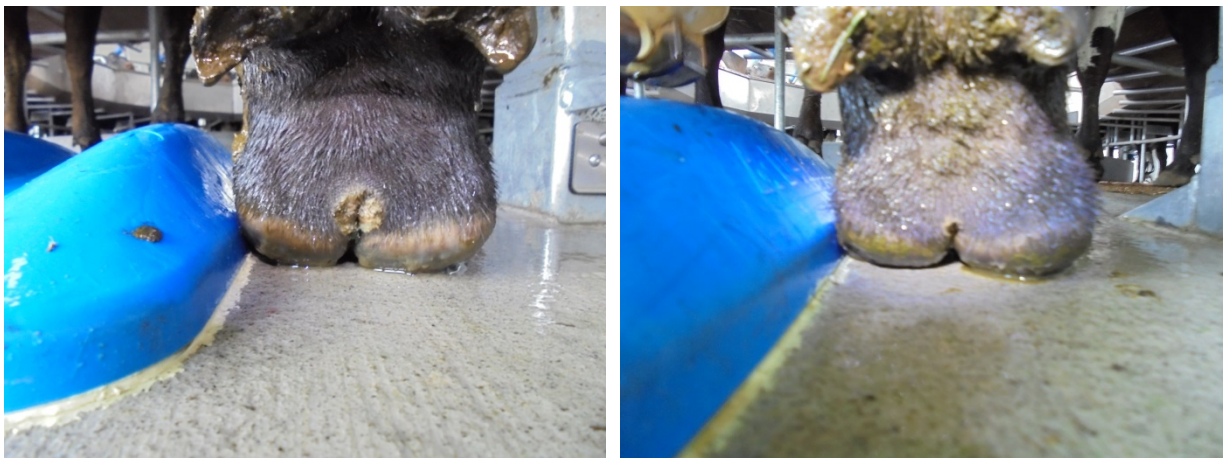

Figure 1. 1 Lesions at the plantar aspect of the foot of two cases: a chronic, inactive lesion (left) and a healing lesion (right).

Aetiology and risk factors of BDD are not well understood in New Zealand. Overseas investigations suggested that Treponema spp. were probably the major aetiological infectious agents in BDD lesions. Five Treponema spp. phylogenetic groups were isolated from BDD lesions in Germany using cloned bacterial 16S rRNA genes (Choi, Nattermann, Grund, Haider, & Gobel, 1997). Three of these groups, described as Treponema medium-like, Treponema phagedenis-like and Treponema denticola-like spirochaetes, have also been isolated in UK and US (Evans et al., 2008; Stamm, Bergen, & Walker, 2002; Walker, Read, Loretz, & Nordhausen, 1995). Strong association between these Treponemea groups and

BDD lesions in the UK and Germany were described by subsequent molecular epidemiological studies (Evans et al., 2009; Nordhoff, Moter, Schrank, & Wieler, 2008). These studies hypothesised that season may be an important risk factor role for lesion development in some affected countries. Differences in climate, housing and management were also noticed (Radostits OM, Gay CC, & Blood DC, 2000).

Much of this information remains unobserved in New Zealand, and there is currently no reliable information on how widespread BDD lesions may be in dairy cows. Therefore, a cross-sectional study was conducted in the Taranaki region to investigate the prevalence and risk factors of BDD.

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2. Herd and Cow Level Prevalence of Bovine Digital Dermatitis of Dairy Cattle in Taranaki, New Zealand

2.1 Abstract

The aim of this cross-sectional study was to estimate baseline prevalence of Bovine Digital Dermatitis (BDD) in the North Taranaki. From September 2014 until February 2015, the feet of 60,455 cattle from milking herds on 224 farms were inspected using a standardised visual inspection method. Data on BDD lesions, feet involved, and type of lesion were recorded. BDD lesions were observed on 143 of the 224 study farms (63.8%). The within farm prevalence varied from 0% (81 farms, 36.2% of total herds) to 12.64% (1 farm). The most common within farm prevalence was over 0% but no more than 3% (53.5% of total farms). The cow level BDD prevalence was 1.2% (95% CI: 0.9% - 3.0%) within 6 study periods. A Bayesian latent class model was designed to predict the true cow level prevalence given priors of sensitivity and specificity of visual inspection based on an expert opinion. The posteriors of within farm prevalence were dependent on the priors of sensitivity and specificity. The posterior of sensitivity was totally dependent on the prior of sensitivity, however, the posterior of specificity 99.9% (95% PI: 99.8% - 99.9%) was not driven by any priors. This study illustrated that BDD was widespread in dairy farms in the North Taranaki, but there were only sporadic cases within a farm that appeared to have a more chronic aspect. It also indicated the visual inspection may have low chance of false positives.

2.2 Introduction

Bovine Digital Dermatitis (BDD) is a contagious foot disease which has been reported in Europe (Blowey, Done, & Cooley, 1994; K. Frankena, E. N. et al., 1991; Holzhauer et al., 2006; Murray et al., 1996; Smits, Frankena, Metz, & Noordhuizen., 1992; Somers, Frankena, Noordhuizen-Stassen, & Metz, 2005a), North America (Cramer et al., 2008; Rodriguez-Lainz, Melendez-Retamal, Hird, & Read, 1998; Rodriguez-Lainz, Melendez-Retamal, Hird, Read, & Walker, 1999; RodriguezLainz, David, Carpenter, & Read, 1996; Wells, Garber, & Wagner, 1999), Iran (Nowrouzian, 1990) and Japan (Kimura, 1993). In many affected countries, BDD was described as a significant reason resulting in lameness (Dhawi, Hart, Demirkan, Davies, & Carter, 2005; Klitgaard, Boye, Capion, & Jensen, 2008; Wells et al., 1999). Due to its

negative impact on economy and animal welfare, many researchers started to investigate its prevalence since it was firstly described.

A cross-sectional study in Netherland found 8.1% of the population during pasture period and 13.8% of the population during housing period were affected with BDD in 1991 (K. Frankena, E. N. et al., 1991). One year later, a prevalence of 17.6% in zero-grazing system was reported in the Netherlands (Smits et al., 1992). Another cross-sectional study suggested that the cow-level prevalence of BDD was 27.3% in the pasture period and 28.5% in the housing period (Somers et al., 2005a). This shows a growing trend over the ten-year period, no matter what system was. In addition, the prevalence of BDD in pasture-based system increased more than 3 times than a decade previously; this could be a negative indication for New Zealand dairy industry. In 2006, a prevalence-focused study done in the Netherlands suggested cow-level prevalence was 21.2% (SE = 0.3). The within herd prevalence varied from 0.0% to 83.0%. Only 9.1% of herds in study were free of disease. The most common within-herd prevalence ranged between 5% and 10% which was approximately 15% of total herds (Holzhauer et al., 2006).

Consistent findings were also demonstrated in North America. A study in 1998 reported 43.5% of US dairy herds were affected by BDD in milk cows in the last 12 month. In the diseased cows, the prevalence was 11.9% of cows and 4.2% of bred heifers. There were 81.9% of cows and 85.9% of bred heifers reported lameness observed in BDD-affected cattle (Wells et al., 1999). Nearly 10 years later, a study concentrating on the prevalence of a variety of foot lesions demonstrated that BDD was the most common foot lesion. In tie stall herds, a cow-level prevalence of 9.3% and a herd-level prevalence of 69.7% were found; the highest within-herd prevalence was 59.5% with an average of 9.6%. Free stalls had a higher cow level prevalence of 22.9% and 92.1% of herds were affected by BDD; the average within herd prevalence was 24.4% with the highest prevalence 66.7% (Cramer et al., 2008). Studies in Chile, which is considered to have a comparable dairy system to New Zealand, illustrated BDD prevalence at the end of last century. A study covering the central and south region of Chile in 1996 suggested 10.5% of cows suffered from BDD using a screening method. For a total of 43 farms in the study, the median within herd prevalence was 6.1%, with a range from 0% to 44.3%; 4 herds were free of this disease. Most of the affected farms had 5% to 10% within herd prevalence, which was 65% of the total herds in the study (Rodriguez-Lainz

et al., 1998). In the same year, the other cross-sectional study enrolled 22 farms out of 32 randomly selected farms in South Chile from previous study. In that study, 11.3% of cows were diagnosed with BDD. The median within herd BDD prevalence for milking cows was 7.4%, with a range from 0% to 40%. Only two farms were free of BDD (Rodriguez-Lainz et al., 1999).

Although many overseas researchers have investigated BDD, the disease has not been well controlled, and understanding of the mechanisms of spread is still poor. The incidence of BBD was still showing an increasing trend in recent years (Brandt et al., 2011). A possible reason was that they started to investigate BDD when it was already spreading epidemically. The prevalence of BDD in New Zealand is not evident, but sporadic cases mentioned from 2004 to 2011 in the first chapter indicated that BDD may be a real emerging disease. Learning from European and American studies may help prevent its spread on an extensive scale in New Zealand. The objective of this pilot study is to investigate the prevalence of BDD in the North Taranaki dairy population to present reliable information of BDD in this country at early stage.

2.3 Material and methods

2.3.1 Study Design and study population

The study consisted of screening for BDD lesions of rear feet on study farms, a survey of herd management, and histological analysis of lesion biopsies to confirm that the farms from which lesions were taken were truly positive for BDD. The study was conducted in the North Taranaki region. The target population was cows from all dairy farms (about 450 dairy farms) in that area. The source population was cows from all the clients (314 dairy farms) registered under Energy Vets, which was a convenient (non-probability) sampling method. The study population was every cow from the farms which agreed to take part in the study. Cow level data such as lesion status and the ID of diseased cows were collected by clinical inspection (screening) during milking time. Two farms were inspected per day. Farm level variables were collected by delivering questionnaires to the study farms. The locations of all the farms involved in the study were pinpointed in Google maps to generate the longitude and latitude. A map of the Taranaki was drawn to indicate the locations of farms sampled.

2.3.2 Visual inspection for BDD

The visual inspection was carried out from September 2014 until February 2015. The farms in the north part of study area (farms near or in Waitara) were selected to be inspected at the beginning of the study as the cows in this region calved earlier than cows in the southern part of the study area (farms near Inglewood). Within each area, the sequence of inspecting farms was randomised. At the beginning of the inspection, Neil Chesterton, the director of Energy Vet, Megan Moss the technician working for Energy Vet and the first author inspected 9 farms together to ensure standardisation of diagnostic criteria and methodology. After this, the technician inspected the milking herds on two different farms to collect cow level data per day, 6 days a week. The visual inspection was performed during the morning and afternoon milking. To prevent the examiners spreading disease from farm to farm, a strict disinfection regime was required before visiting any farm.

During inspection, every cow's rear feet were carefully cleaned by water hose, after which a head torch was used to observe the feet clearly. Only the rear feet of cattle were inspected due to practicalities; this was considered reasonable as the international literature indicates that lesions are very rarely seen on front feet (Murray et al., 1996). Lesions were described according to gross appearance and the location. The lesions were divided to 3 categories: activated lesions, dried lesions and suspicious lesions (healing lesion or uncertainty). The case definition was that a cow had at least one erosive, light grey or brown, granulating lesion, exuding an area with mottled hair (dried lesion) or flat, red, bleeding lesion (activated) at the back of the foot without swelling in the adjacent tissue. Cows with BDD lesions could be very sensitive when its foot (or feet) were touched by examiners' finger or cleaned by pressurised water flow. Colour pictures of lesions were taken in some randomly selected herds to discuss the recognition of lesions with other researchers involved in this study. For every cow with lesion(s), its identification number (ID), feet involved, and type of lesion(s) were recorded. No information was recorded for cows without lesions. These records were the only cow-level information available. For some large lesions (diameter >3 cm), the cows were marked and lesion samples were taken and sent to the laboratory. Data on herd size, number of bails, row serial number and type of milking platform (herringbone or rotary) were also recorded. To avoid disrupting the normal milking routine, the time spent inspecting cows in a row or rotation was minimized in order to avoid disturbing the

cow environment and allow for routine milking to process as usual. Approximately two-thirds of the farms were inspected by the technician only; the remaining farms were inspected by the technician and the first author together.

2.3.3 Survey of herd managers

This procedure was aimed at understanding the risk factors which will be described in later chapter. The questionnaire designed by BDD Working Group, which includes representatives from Massey University, the Ministry for Primary Industries, private veterinary practice (Energy VET), the Dairy Cattle Vets branch of the New Zealand Veterinary Association and DairyNZ was delivered to every farm inspected. The questionnaire contains colour photos, background of BDD, aim of the survey, farm details, farm level variables, previous BDD observations by workers, hoof care and farm management (available from the authors if required). It also stressed the importance of completing the survey and guaranteed confidentiality. It was preferred to deliver the questionnaires to farm owners or anyone who was responsible for the herd after herd inspection. While delivering the questionnaire, a thanks letter and a bag of chocolate were also delivered to encourage the farm owners or share milkers to respond in time. Meetings held by Neil Chesterton several times aimed to explain the significance of survey and benefits received from the study. A sheet including fact description and instruction of treating and preventing BDD was also delivered to every affected farm. This procedure was mainly for risk factors analysis; the results of this procedure would be presented in the next chapter.

2.3.4 Biopsy of BDD lesions

This procedure was to confirm BDD on farms found positive by visual inspection. A few selected lesions regarded as being typical for BDD for sent to either of two laboratories, Gribbles Veterinary Pathology Ltd. and New Zealand Veterinary Pathology (NZVP), for histo-pathological examination. The criterion of biopsy was size-dependent. If the diameter of lesions was over 3 cm, the cows were marked and lesion samples were taken and sent to the laboratory. If the laboratory results confirmed the diagnosis of BDD from visual inspection, the farm was regarded as being positive. If BDD was not confirmed, a second sample from the same farm was sent for histo-pathological examination. Two suspicious cases from a negative farm were also sent to the laboratory (NZVP). The aim here was trying

to confirm our case definition 'suspicious' was correctly diagnosed as 'not-BDD' as suspicious lesions were not counted as BDD cases. The two laboratories used the same histological scoring technique to characterise the lesions and confirm or rule out BDD.

2.3.5 Data Analysis

Data validity for prevalence dataset

There were 3 datasets stored in "Google drive" including (i) farm level information, (ii) cow level information from clinical inspection, and (iii) farm level risk factors collected by questionnaire. The farm information sheet recorded numbers of diseased cow, herd size, date of inspection and type of milking shed in each farm. The cow information sheet recorded the ID of diseased and suspicious diseased cows, their involved feet and farm ID. For the farm level data, missing values were checked at first. Secondly, to make sure the prevalence was accurate; the number of diseased cows from both cow information sheet and farm information sheet had to be the same. To validate the data, the number of diseased cows used for modelling prevalence was compared between cow and farm information sheets. To ensure consistency of the number of diseased cows, the counts of diseased cows from both sheets were adjusted if a difference was found. Hard copies of records after inspection were checked to understand the reason of different counts in both sheets. Only credible true cases numbers were used after these validation steps.

Farm and cow level prevalence

From the cow information sheets, counts of cows with right hind foot lesion, left hind foot lesion, both hind feet lesions were recorded to compare if the lesions were evenly seen from different feet. The prevalence included within farm prevalence (number of diseased cows divided by herd size in each farm), between farm prevalence (numbers of affected farms divided by total number of farms) and overall cow level prevalence in study population (number of diseased cow from all farms/number of cows from all farms). Farm level prevalence for the whole study period was calculated directly. Overall cow level prevalence in the study area was fitted by an intercept only Generalised Estimating Equations (GEE) model (Liang & Zeger, 1986) by Stata/IC 13.1 using robust standard errors to adjust for within farm correlation. The significance level was set at P=0.05. A map which

described the disease status was generated and combined with the map of the sampling area.

Descriptive statistics of results from biopsy

Descriptive statistics were used for biopsy data. Several approaches were used. Firstly, the number of total samples taken and number of farms selected were compared to detect whether or not there was more than one sample coming from a certain farm. Secondly, number of tested farms stratified by different laboratories was examined. Thirdly, numbers of positive cases from both screening and lab reports were checked. Fourthly, the data were summarised by two 2X2 tables for counts of lab results versus screening results stratified by laboratories. Finally, lab results versus laboratories were cross-tabulated.

Bayesian latent class model to predict the true prevalence

Apparent BDD prevalence calculated from visual inspection may be biased due to misclassification errors. The misclassification error can be explained by the imperfect visual inspection. Hence the true BDD prevalence would be produced after adjusting for the sensitivity and specificity of the visual inspection. The “true” prevalence was correctly calculated only if the sensitivity and specificity were correctly defined (e.g. a “gold standard” test is available). When considering histology as a gold standard, a random subset of samples from visually typical, suspicious and negative cows would have to be collected and subjected to histo-pathological examination. However, only samples from typical lesions of selected cows were submitted to a laboratory and no non-typical BDD lesions or tissue from visually unaffected cows were sampled and examined by histo-pathological examination. Therefore, the false negative rate could not be estimated by the gold-standard method from available tissue samples. Therefore, gold standard methods could not be used and a Bayesian latent class approach was chosen for predicting the true BDD prevalence.

Bayesian latent class models are based on the frequentist 2-test/2-population model (Hui & Walter, 1980). Bayesian latent class models were described by Johnson W.O. to estimate the sensitivity and specificity of diagnostic tests (Johnson, Gastwirth, & Pearson, 2001). The model was modified to a model with two-test and multiple populations with random prevalence (T. Hanson, Johnson, & Gardner, 2003). The Hanson et al. model was modified to

allow for herds with zero prevalence (Branscum, Gardner, & Johnson, 2004; T. E. Hanson, Johnson, Gardner, & Georgiadis, 2003; Johnson, Su, Gardner, & Christensen, 2004; Stringer et al., 2013). We applied these methods to predict the true prevalence given one test (visual inspection) on multiple populations where some of the farms were possibly not BDD infected.

Visual inspection had a binary outcome for each cows (positive/negative). In a certain farm, the number of positive cows "x" follows a binomial distribution. "x" ~ binomial (p, M), where p is the probability of visually diagnosing BDD in a cow, and M is the number of cows inspected (Branscum et al., 2004). When we have multiple farms "k", then x [k, 1] ~ binomial (p[k], M[k]) is the number of cows with BDD on farm k. To a random farm k, the relationship between apparent prevalence p[k] (proportion of cows positive for BDD by visual inspection), true prevalence, sensitivity and specificity of screening can be summarised as following equation:

$$p[k] = \pi[k] \times \eta + (1- \pi[k]) \times (1-\theta)$$

Where $\pi[k]$ means the true prevalence of BDD within farm k; η and θ means the sensitivity and specificity of the visual inspection method, respectively.

The true prevalence for a random farm k can be zero if the farm was free of BDD. Therefore, the model allowed for farms with zero prevalence as follows:

$$\pi[k] = z[k] \times \pi^*[k]$$

Here the true within farm prevalence $\pi[k]$ is divided into herds with zero prevalence (for all z[k] = 0) and herds with prevalence $\pi^*[k]$ if z[k]=1; z[k]follows a Bernoulli distribution of between farms prevalence (θ).

Thus, we have z[k] ~ Bernoulli (θ).

For farms with lesions, the within farm prevalence (π^*) was fitted with an intercept only random effect logistic regression model:

$$\text{Logit}(\pi^*[k]) = \beta + \mu[k]$$

Where β is the intercept, i.e. overall logit-mean within herd prevalence and μ[k] means the random effect in a random infected farm which follows a normal distribution at the logit-level: "μ[k]" ~ Normal (0, τ), where the software models τ as precision of the random effect (=1/variance).

As this was a pilot study, there were no other reliable data suggesting the prior information of visual inspection performance, median within farm prevalence and between farm prevalence. Thus, expert opinion was used for defining priors. Neil Chesterton was interviewed by the first author to obtain the information for priors. Considering the pilot study, no other researchers could estimate the prevalence of BDD in this area except Neil Chesterton, because he started to investigate the disease in the Taranaki two years ago before this study. Besides, Neil Chesterton understood the technician's ability to identify the lesions because they worked together to standardise the criteria of BDD lesions in the nine farms at the beginning of screening as mentioned previously. To ensure his ideas were objective, the priors were determined before data analysis. This means Neil Chesterton was unaware about prevalence of BDD from this study when suggesting the priors. Besides Neil Chesterton's suggestion, a published paper summarised that visual inspection of BDD in Chile had a sensitivity of 0.72 (95% confidence interval (CI): 0.53-0.86), and a specificity of 0.99 (95% CI):0.93-0.99) (Rodriguez-Lainz et al., 1998).

We considered the sensitivity of visual inspection may be very different in NZ because lesions may not be as clear in NZ as in confined system. We therefore would specify a pessimistic prior with large uncertainty for the sensitivity. This resulted in the most likely prior estimates for sensitivity and specificity of visual screening of 50% and 95%, respectively. The expert was 95% sure that the sensitivity was greater than 30% and specificity was greater than 85%, corresponding to beta distributions with parameters a= 8.0011 and b= 8.0011 for sensitivity, and a= 36.7028 and b= 2.8791 for specificity. The median within farm prevalence was assumed to be 7% and the expert was 95% sure that it was over 1%. The intercept β from logit function followed a normal distribution. The precision τ for the random followed a Gamma (1, 1) distribution. Diffuse prior Beta (1, 1) was defined for the proportion of BDD positive farm. The priors and their corresponding distribution parameters are summarised in Table 2.1.

Table 2. 1 Prior information on between and within farm prevalence, screening sensitivity and specificity for Bayesian latent class model.

Prior	Estimate	95th Percentiles	Distribution
Between farm prevalence			Beta (1, 1)
Within farm prevalence	7%	>1%	Logit-normal (-2.5867, 0.67084)
Sensitivity	75%	>55%	Beta (8.0011, 8.0011)
Specificity	95%	>80%	Beta (36.7028, 2.8791)

Sensitivity analyses were conducted to assess to what extent posterior distributions depended on priors or data, using optimistic and pessimistic priors for the sensitivity by increasing and decreasing sensitivity by 10%, pessimistic prior for specificity by dropping 15% and a wider prior for the within farm prevalence (median=15%, 95% sure >1%).

The model was developed in OpenBUGS (version 3.2.3 rev 1012). The three chains were thinned by 5 to reduce autocorrelation and running for 30,000 iterations, after discarding a burn-in period of 8,000. The model convergence was assessed by history plot and BRG-diagnostics plot; the auto-correlation was assessed by auto-correlation plots.

2.4 Results

2.4.1 Study population and screening for BDD

There were 224 farms including 60,455 dairy cattle involved in the study. Only 4 farms refused to join. The reason of not inspecting other available farms was that more time was focused on revisiting the farms, which was discussed in BDD working group meeting in February 2015. The affected cows normally reacted sharply, kicking the involved shank away strongly. The lesions which were considered as positive and suspicious (not counted as cases) were shown in Figure 2.1.

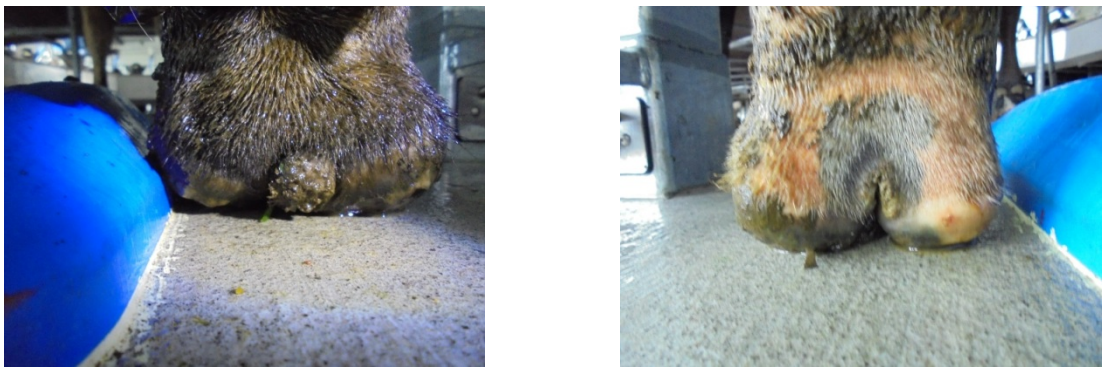

Figure 2. 1 Lesions inspected in a random selected farm during screening stage: a positive lesion (left) and a suspicious lesion (right).

2.4.2 Biopsy of BDD lesions

There were 47 samples from 42 different farms sent to 2 different laboratories (Gribbles and NZVP). Gribbles tested samples from 20 farms while NZVP tested samples from 22 farms. Among 42 farms, 5 of them sent second samples to undertake histo-pathological examination because the previous test results suggested samples from those farms were negative. In those 5 samples, two of them tested by Gribble were identified as positive; three of them tested by NZVP were identified as negative still. One clean farm had 2 cows with suspicious lesions which were not counted as cases. Samples from those 2 cows were both sent to NZVP (the farm is a twice-tested farm), the results of two times both suggested they were negative lesions. As mentioned if there was any sample identified positive, the farm was confirmed to be truly affected by BDD. Therefore, pooling the results from both first time and second time tests was of interest in farm level infection status. Out of 42 farms, 27 farms were tested positive by histo-pathological examination, while 41 out of 42 farms were found to be affected farms by screening. There was substantial disagreement between laboratories (Table 2.2). As mentioned, Gribbles did 20 samples by histology test and reported that 19 out 20 were positive; NZVP did 22 samples and reported that 8 samples were positive, even though they used the same technique.

Table 2. 2 2X2 table of histology results of bovine digital dermatitis lesions of farm level in the North Taranaki by 2New Zealand Veterinary Pathology and Gribble Veterinary

		Histology conclusion of lesions tested		
		Positive	Negative	Total
Laboratories Involved	NZVP	8	14	22
	Gribble	19	1	20
				42

2.4.3 Data Validity for prevalence dataset

There were no missing values in both farm information sheet and cow information sheet among those farms. There were 710 cases in farm information sheet but 707 cases out of 927 records (the others were suspicious cows) in cow information sheet. After several examinations of inconsistent records of case numbers, Farm 1 and Farm 2 had 2 more records respectively in farm information sheet than cow information sheet, while Farm 16 had 1 less record in farm information sheet. The reason here was that in those farms, a few

cows were recorded twice in hard copies, after the typing into the sheet; cow information sheet contained those repeated records which were wrong. Therefore, the farm information sheet used the wrong counts produced from cow information sheet. After checking the cow level information sheet, the repeated records were deleted, but the farm information sheet was not updated. That was why the counts of cases disagreed with each other. The true cases used in further analysis were 707.

2.4.4 Farm and cow level prevalence

There were 143/224 (63.8%, 95%CI: 57.5% - 70.1%) farms were diagnosed with BDD by screening. The within farm prevalence varied from 0 (81 farms, 36.2% of total farms) to 12.7% (1 farm). The most common within farm prevalence was over 0 but no more than 3% (53.5% of total farms). Results were summarised in Figure 2.2. The information including location and farm affected status of all the farms involved in the study is shown in Figure 2.3. The overall cow level prevalence of all the farms during the whole study period was 1.2% (95%CI: 0.9% - 3.0%) in this cross-sectional study. Results were summarised in Table 2.3. The prevalence of BDD did not vary much between both hind feet. In 707 cases, 37.9% of cows had a lesion on left foot only, 37.1% of cows had a lesion on right foot only and 25.0% of cows had lesions on both feet.

Table 2. 3 Summary of farm and cow level prevalence in 60455 cows from 224 farms in the North Taranaki.

Parameters	**Counts or Prevalence**	**95% CI**	**Comments**
Number of farms	224		
Number of BDD positive farms	143	57.5% - 70.1%	positive farms/total farms
Between farm prevalence	63.8%		
Number of cows	60455		
Number of cows in positive farms	41116		
Number of BDD positive cows	707		
Cow level prevalence in all farms	1.2%	0.9% - 3.0%	positive cows/total cows
Cow level prevalence in positive farms	1.7%	1.4% - 2.1%	positive cows/cows in positive farms

CI: confidence interval.

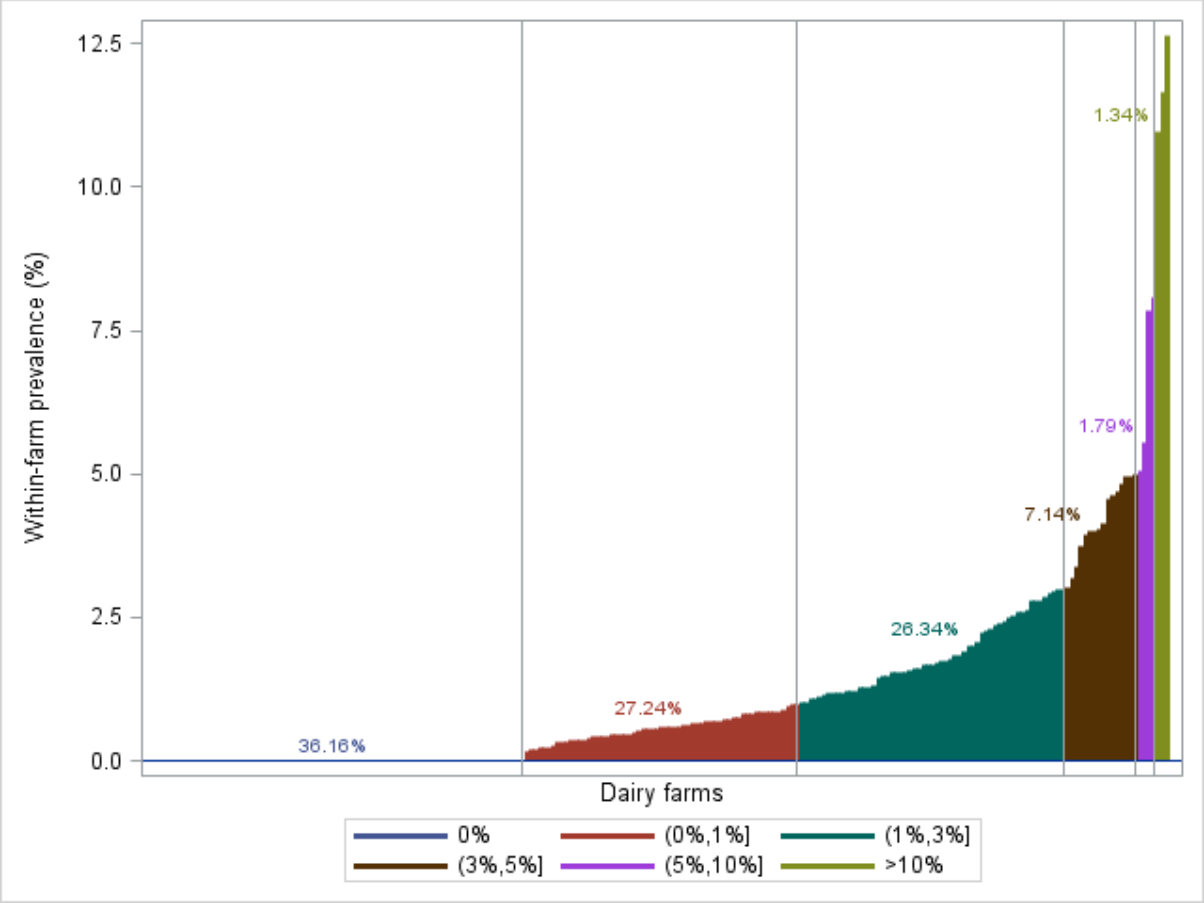

Figure 2. 2 Within farm prevalence of bovine digital dermatitis in 224 farms (60455 dairy cows) in The North Taranaki.

2.4.5 Outputs of Bayesian latent class model

There was no evidence of shortage of convergence of the model suggested by history and BGR-diagnostics plots. The auto-correlation was not of concern suggested by auto-correlation plots. The predicted average within herd prevalence in infected herds was 2.1% (95% probability interval PI: 1.2% - 4.3%). In the sensitivity analysis, the average within herd prevalence was driven by the priors of sensitivity and specificity. The prevalence decreased by increasing the prior of sensitivity; the prevalence increased by decreasing the prior of specificity. However, the prevalence was not dependent on its priors. The posteriors of sensitivity were entirely dependent on the priors of sensitivity. However the specificity 99.9% (95% PI: 99.8% – 99.9%) of visual inspection estimated by Bayesian latent class model were not driven by any priors. The estimated values and 95% probability intervals were summarised in Table 2.4.

Table 2. 4 Results of Bayesian latent class model and sensitivity analyses of the average within herd prevalence, sensitivity and specificity of visual inspection for bovine digital dermatitis of dairy cattle in the North Taranaki.

Parameter	Median	SD	2.5% quartile	97.5% quartile
Model based on expert opinion				
Prevalence	2.1%		1.2%	4.3%
Sensitivity	0.473	0.1192	0.249	0.707
Specificity	0.999	0.0006	0.998	0.999
Sensitivity Analysis using pessimistic prior for screening sensitivity				
Prevalence	2.5%		1.3%	5.3%
Sensitivity	0.387	0.1233	0.187	0.662
Specificity	0.999	0.0006	0.998	0.999
Sensitivity Analysis using optimistic prior for screening sensitivity				
Prevalence	1.8%		1.0%	3.4%
Sensitivity	0.561	0.1168	0.328	0.781
Specificity	0.999	0.0006	0.998	0.999
Sensitivity Analysis using pessimistic prior for screening specificity				
Prevalence	2.6%		1.4%	5.6%
Sensitivity	0.479	0.1224	0.256	0.723
Specificity	0.997	0.0004	0.996	0.998
Sensitivity Analysis using median within farm prevalence = 15%, 95% sure >1%				
Prevalence	2.1%		1.1%	4.2%
Sensitivity	0.470	0.1202	0.251	0.713
Specificity	0.999	0.0006	0.998	0.999

2.5 Discussion

2.5.1 Prevalence of BDD

The aim of this cross-sectional study was to understand the natural prevalence of BDD in the North Taranaki when few vets and farmers paid attention on it. Compared to many overseas publications, the prevalence of farms (64%) and cows (0 – 13%) of BDD in the North Taranaki area was substantially lower. The Netherlands and Chile both reported 90.9% farms were infected by BDD. The within farm prevalence varied from 0 to 83% in The Netherlands (Holzhauer et al., 2006) and from 0 to 44% in Chile (Rodriguez-Lainz et al., 1998). A study in US reported that a cow-level prevalence of 9.3% and a herd-level prevalence of 69.7% were found in tie stall herds and a cow level prevalence of 22.9% and 92.1% of herd prevalence were found in free stall herds (Cramer et al., 2008). In our study, there were many lesions reported as suspicious which were not included in the case count. Therefore, the cow level prevalence could have been underestimated. The percentages of

lesions found in left and right hind feet were similar, which was consistent with some overseas' publications (Holzhauer et al., 2006; R. A. Laven, 1999; Rodriguez-Lainz et al., 1998).

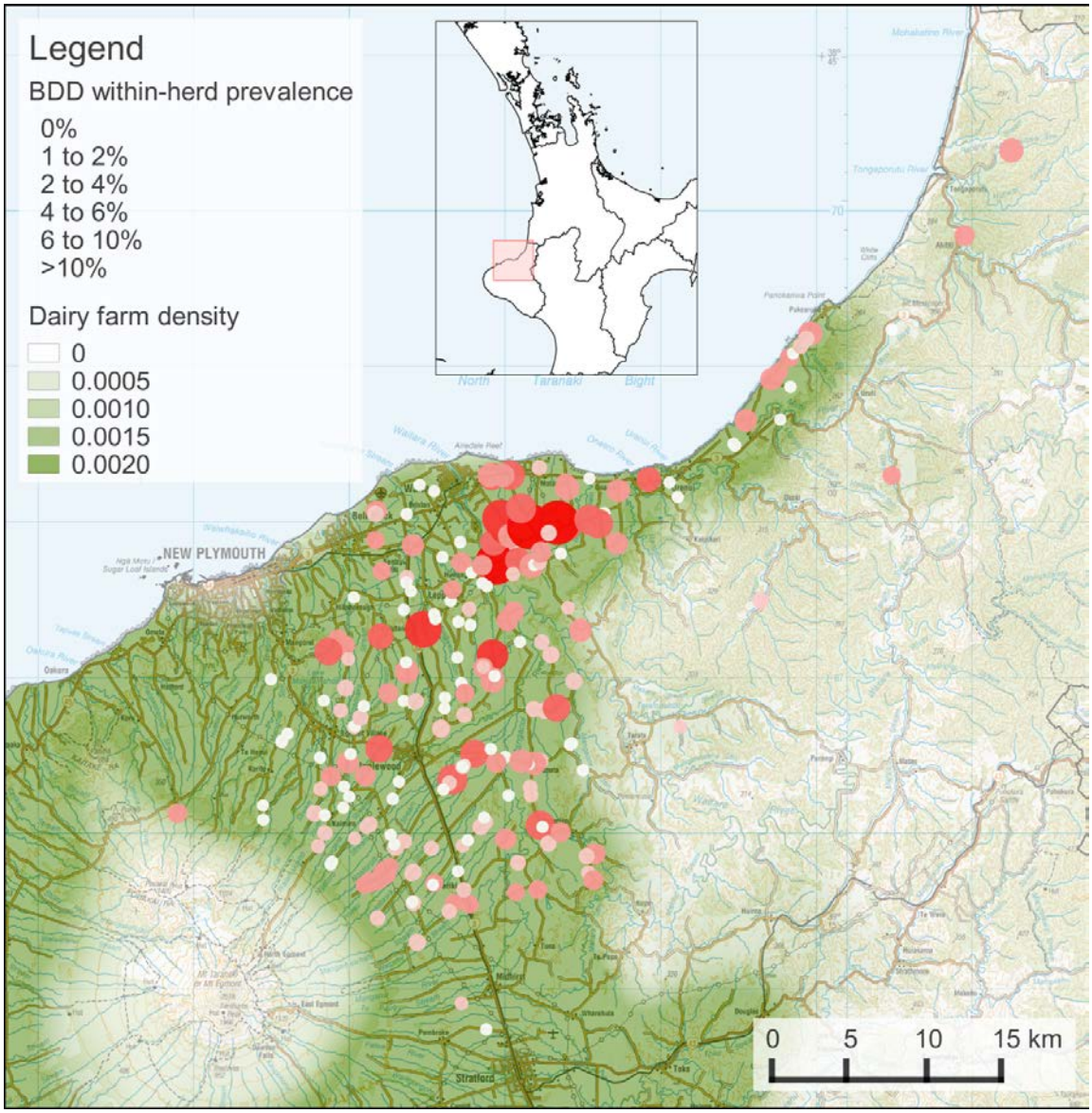

Figure 2. 3 Farms' locations and within-herd prevalence in the North Taranaki. The size and colour of the points is proportional to the prevalence. The green shading represents density of dairy farms (source: FarmsOnLine). Inset: overview of the study area. BDD: bovine digital dermatitis.

2.5.2 Study population

This screening covered about 50% (224/450) of dairy farms in the North Taranaki lasting 6 months. Due to the convenient sampling method, the dairy farms around New Plymouth were not included in this study. The disease-status showing map illustrated that the disease was randomly distributed and the distance between the study area and New Plymouth

District were not far away from each other. The actual participant rate in this study was 71.3% (224/314). As mentioned before, a higher participation rate can certainly be achieved, only 4 farms declined to participate in this study. However, the prevalence is unlikely to have been very different if more farms were inspected. Therefore, for this study, the visual inspection was stopped in February 2015 when the issue was discussed in the BDD working group meeting. The convenient sampling method here had the obvious benefit that a very low refusal rate 1.8% (4/228) was achieved which minimised selection bias. A caveat existed in that none of the dairy farms around New Plymouth were enrolled in the study; hence our estimates are not valid for the entire population of dairy farms in the Taranaki region.

During project planning, a systematic random sampling method that picks 2 cows at the first row, 2 cows in the middle row, and 2 cows in the end row was considered. Recording row ID was used in the inspection stage. However, eventually this method was not adopted because often in a rotary shed some cows remain on the milking platform for more than one rotation (this actually happened). Moreover, healthy cows staying on the milking platform for more than one rotation would be inspected again because cow IDs of negative animals were not recorded. This will affect the apparent prevalence by overestimating the denominator. Therefore, a systematically random sampling method might have generated biased prevalence estimates when applied in rotary herd.

2.5.3 Visual inspection

Visual inspection could be applied only when milking cows came to the shed. To avoid a disturbance of the normal milking routine and to make sure our observations under normal environment, time spent on inspecting cows in any farm was minimized. The prevalence could have been underestimated because limited time was spent for BDD inspection and scoring. Firstly, lesions could have been missed in the early or late stages of development when lesions were frequently small and difficult to see, particularly when the water flow was not strong enough to clean the feet completely. Secondly, lesions were frequently masked from sight if they were on the bottom of the feet because the limitation of time did not allow us to lift any cow's feet. Due to the reasons mentioned, the prevalence of the condition on any farms was likely underestimated.

2.5.4 Biopsy of BDD lesions

In this study, histo-pathological examination was used to confirm BDD in lesions found by clinical inspection to be true positive. If one lesion was identified as positive lesion by histo-pathological examination, the farm from which the sample came must be positive farm. The only two “negative” samples (by case definition in this study) sent to the laboratory were suspicious (non-conclusive) lesions as one of the researchers wanted to know how the histo-pathological examination defined the suspicious lesions from clinical inspection, hence these were not meant to be used as control group to validate the laboratory results. However, the significant disagreement between two laboratories raised concerns, especially because they used the same histo-pathological technique and scoring system.

The agreement of histo-pathological examination and visual inspection could not be evaluated due to the lack of a visual test negative control group. Besides, the performance of visual inspection (sensitivity and specificity) was hard to be evaluated either. In a meeting with the pathologists of the two laboratories, the reasons for the disagreement were discussed. NZVP suggested that the samples were regarded as being negative when stratum corneum was not completely lost. In contrast, Gribbles treated them as positive if this was observed. Photos of the samples sent to the laboratories were further discussed in a BDD working group meeting with overseas experts Dr. Read and Associate Professor Döpfer. Both considered clinical positive samples without completely loss of stratum corneum to be true positive.

2.5.5 Bayesian latent class model

Bayesian latent class model could be used to predict the true prevalence when single test was applied on multiple herds. In this model, the degree of freedom concept was not clear because of assuming the within farm prevalence is a random effect; so instead of 223 prevalence parameters, there were two parameters to describe the distribution of the prevalence.

The likelihood part of model was not identifiable, the priors may dominate the posteriors; therefore informative priors were essential to produce the reliable posteriors. The estimated prevalence could be true only if the defined priors for the sensitivity and specificity were correct. Considering lack of reliable priors, we allowed large uncertainty in

priors. We also performed the sensitivity analysis to examine what extent posterior distributions depended on priors or data. Although the posteriors of specificity in every scenario were constant, the posteriors of sensitivity were driven by the priors of sensitivity. It was difficult to conclude the sensitivity of the visual inspection. It was therefore difficult to estimate the true prevalence given unreliable sensitivity. However, the high specificity of visual inspection could be concluded because the posteriors of specificity were not driven by any priors. That could be explained that the chance of false positive must be low when the data contained a lot of results with zero apparent prevalence.

2.6 Conclusion

The cow level prevalence of BDD in the North Taranaki was low, but the between farm prevalence was high. Eradication strategy of BDD in that area could be considered due to the low cow level prevalence. The prevalence of BDD in other areas of New Zealand is still unknown; a study which could cover more regions or even nation-wide would be desirable. An appropriate sample size could be considerably smaller, but should examine more tissue samples by histo-pathological examination for more accurate diagnosis. The histo-pathological examination of tissue samples from cows negative on visual inspection would enable to investigate the sensitivity of inspection. Laboratories should adopt the same criteria for diagnosing BDD from lesions tissues. Bayesian latent class model suggested the visual inspection had a high specificity; however it did not make solid inference on the sensitivity and true within herd prevalence.

2.7 Acknowledgement

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3. Herd Level Risk Factors of Bovine Digital Dermatitis in Tarankai, New Zealand

3.1 Abstract

The aim of the study was to identify risk factors of bovine digital dermatitis (BDD) in the North Taranaki, New Zealand (NZ) from September 2014 to February 2015. Farmers completed questionnaires about herd structure, management and biosecurity related factors on the day of inspecting the milking herd. The outcome was the counts of BDD positive cows within each farm. The data were analysed by fitting a generalised linear model with negative binomial distribution and log link. The model suggested that more cases were found in rotary than herringbone sheds (risk ratio (RR)=1.70; 95% confidence interval (CI): 0.95-3.05). Compared to September, fewer BDD cases was detected in October (RR=0.41, 95%CI: 0.19-0.91), December (RR=0.19, 95%CI: 0.08-0.45) and January (RR=0.38, 95%CI: 0.17-0.85). However, counts of BDD cases in November (RR=0.56, 95%CI: 0.24-1.29) and February (RR=0.59, 95%CI: 0.22-1.58) were not significantly different from September. Compared to farms not purchasing cows, farms which purchased cows had higher prevalence of BDD (RR=1.69, 95%CI: 0.98-2.91). The risk of BDD infection was higher in farms which introduced grazing-off heifers (RR=2.06, 95%CI: 1.11-3.83), compared to farms not receiving grazing-off heifers. Farms were at higher risk of having BDD if the farm used external staff (veterinarians) to trim feet (RR=2.28, 95%CI: 1.26-4.13), compared to farms where all foot trimming was undertaken only by farm staff.

3.2 Introduction

Bovine digital dermatitis (BDD) has a moderate long history in the United Kingdom, Netherlands, many other European countries and American continent (Blowey & Sharp, 1988; Cornelisse, Peterse, & Raven, 1981; Demirkan & Guzel, 2004; K. Frankena et al., 1991; Read & Walker, 1998; Rodriguez-Lainz et al., 1999; RodriguezLainz, Hird, Walker, & Read, 1996; Smits et al., 1992; Yeruham & Perl, 1998). However, in New Zealand, the first BDD case was reported in 2004 (Vermunt & Hill, 2004). Since that, sporadic cases were reported to Ministry of Primary Industry. Transmission within New Zealand dairy farms is probably due to buying subclinical animals from infectious herds. In addition, the naïve herds could be infected with BDD by visiting of veterinarians and hoof trimmers who did not disinfect

their facility entirely after working in the infectious herds. But those hypotheses were not verified at the moment. Besides lack of verifying the infection process, the potential risk factors associated with BDD in New Zealand were not understood.

Overseas studies tend to evaluate treatment and aetiology of BDD in most recent years (Brandt et al., 2011; Cutler, Cramer, Walter, Millman, & Kelton, 2013; Schultz & Capion, 2013; Yano et al., 2010), but they investigated the BDD related risk factors in last two decades. Summarily, the herd level risk factors included poor hygiene, housing system, pasture access, herd size, footbath regimen, poor biosecurity and season; the cow level risk factors included parity, lactation stage, production performance and breed.

A case-control study in US compared 37 high incidence farms (annual average proportion of > 5%) as cases to 20 low incidence farms with no more than 5% annual average proportions (controls). In that study, poor hygiene had a huge impact on BDD. If cattle were exposed to muddy environment, they were more likely to be affected by BDD in no heifer-buying farm (OR=19.2, 95%CI: 3.13-118) and heifer-buying farm (OR=22.4, 95%CI: 3.48-144), respectively (RodriguezLainz, David, et al., 1996). Rough floor in terms of housing system was another risk factors identified by many researchers (K. Frankena et al., 1991; Rodriguez-Lainz et al., 1999; Somers et al., 2005a; Wells et al., 1999). There were some inconsistent findings about pasture access. As mentioned in Chapter 2, limitation of pasture access increased BDD prevalence (K. Frankena et al., 1991; Somers et al., 2005a), while in another Dutch cross-sectional study, more than eight hours grazing on pasture had positive association with BDD (Holzhauer et al., 2006). A possible explanation here could be cattle which had had BDD already were exposed to pasture by stockmen on purpose. Herd size was another risk factor with a lot of discussions. Medium herds (50 to 65 cows) was a risk factor for BDD in Netherlands compared to small herds (K. Frankena et al., 1991), similarly, large herds (over 200 cows) had positive association with BDD (OR=2.7, 95% CI:1.7-4.5) in US (Wells et al., 1999). When treating herd size as continuous scale, odds of BDD increased with increasing herd size (Rodriguez-Lainz et al., 1999). However, another Dutch study suggested there was no association between BDD and herd size when herd size was treated as categorical scale (Somers, Frankena, Noordhuizen-Stassen, & Metz, 2003). A Chilean study suggested foot bath showed protective effect on BDD (Rodriguez-Lainz et al., 1999). Poor biosecurity contains many aspects. Acquiring animals from other herds was reported as a significant risk

factor by many studies (Rodriguez-Lainz et al., 1999; RodriguezLainz, David, et al., 1996; Wells et al., 1999). Comparing to buy adults, buying heifers was more risky (Rodriguez-Lainz et al., 1999). The other aspects of biosecurity were visiting by other people and disinfection. Cattle trimmed by hoof trimmers who also operated on other farms and hoof-trimming equipment not routinely washed with water between cows were both at higher risk (Wells et al., 1999). BDD morbidity also showed a seasonal pattern, late spring and summer were identified as risk period in US (Wells et al., 1999).

In respect of cow level risk factor, Holstein-Friesian was predisposed to BBD compared to other dairy breeds (K. Frankena et al., 1991; Rodriguez-Lainz et al., 1999). Many studies reported a dose response observed that increasing parity would decrease the odds of BDD; the first parity cattle had the highest odds (Holzhauer et al., 2006; Rodriguez-Lainz et al., 1999; Somers et al., 2005a). The association between lactation and BDD had no exact cut-offs. There was no association between lactation stage and BDD, if 30 days and 70 days were used as cut-off; but risk of BDD increased significantly with increasing days of lactation when 90 days, 180 days and 270 days were used as cut-off (Rodriguez-Lainz et al., 1999). Dry cows had a lower risk compared with lactating cows (Somers, Frankena, Noordhuizen-Stassen, & Metz, 2005b). Top production cows were at more risk of BDD possibly due to nutrition aspect which was not intensively studied (K. Frankena et al., 1991).

The aim of this study was to identify farm level risk factors of BDD in the North Taranaki, New Zealand.

3.3 Material and Method

3.3.1 Study Design and data collection

The study design and methodology had been previously described in the last chapter. Briefly, the study consisted of screening for BDD lesions on rear feet of study population to obtain outcome information, surveys of herd management of every farm visited to obtain farm level exposures. The study was conducted in the North Taranaki; the target population was cows from all the dairy farms (about 450 dairy farms) in that area. The source population was cows from all the clients (314 dairy farms) registered under Energy Vet. The study population were cows from 224 farms which finally took part in the study. Cow level data such as lesions and ID of diseased cows and part of farm level data such as herd size and

type of the shed were collected by clinical inspection during milking time 2 farms a day, 6 days a week by the vet technician and the first author from September 2014 until February 2015. A strict disinfection was performed before visiting any farm to prevent the examiners spreading disease from farm to farm. During inspection, every cow's rear feet were cleaned by water hose carefully, and then a head torch was used to help observe the feet clearly. Diagnosis was according to gross appearance and the location. The case definition was defined in the chapter 2. The aim of herd inspection was mainly to estimate prevalence at farm and animal levels and to understand how farm management impacted on the prevalence of BDD.

Farm exposure variables were collected by delivering a questionnaire to farmers. The questionnaire included colour photos of BDD lesions, background information about BDD, the aim of the survey, farm details. In addition, data about the following management variables were collected: number of introduced animals stratified by animal type (bull, heifer, calf), source of purchased animals, mode of transportation, previous BDD observation by workers, hoof trimming including the frequency of performing hoof trimming and if hoof trimming was performed by external staff, and feeding system for both lactating and dry cows. The questionnaire is available from the authors by request. An accompanying letter stressed the importance of completing the survey and assured confidentiality. The questionnaire was administered to farm owner or manager. While delivering the questionnaire, a letter of appreciation and a bag of chocolate were provided to encourage farmers to respond in time. A prepaid envelope was attached with the questionnaire for return by mail.

3.3.2 Building a Causal Model

The causal model is presented as a causal diagram drawn in Daggity (Textor, Hardt, & Knuppel, 2011) to help understand the potential causal network and identify confounders or intervening variables. A confounder would always be included in the regression model regardless of its statistics significance. An intervening variable was only included in the model when interactions were tested between adjacent variables, else intervening variables were removed to allow the model to output the total effect of potential risk factors on BDD.

3.3.3 Statistical analysis

Explanatory Data Analysis

First of all, all the variables in the dataset stored for risk factor analysis were checked if they had missing values or incorrect records. For missing values or some ambiguous records, we contacted the farmer by phone to make sure their answers were accurate. Histograms were produced for all the continuous variables to check their distributions. For the variables which were normally distributed, the mean and standard deviation would be used to describe their central tendency and spread. For those variables which were not normally distributed, the median and quartiles including minimum and maximum values were used to describe the central tendency and spread. For categorical variables, multiple one way tables were produced to check their frequency. If the categorical variables had several categories with low counts, those categories were combined with adjacent categories if this was biologically plausible. Only variables with substantial variability were selected in the analysis (I. Dohoo, W. Martin, & H. Stryhn, 2003b). The variables were initially used in explanatory data analysis shown in Table 3.1.

After univariate screening, the next procedure was correlation analysis. The procedure aimed at identifying variables that contained essentially the same information. If two variables were highly correlated with each other, the one which was considered more important was selected for future analysis (I. Dohoo et al., 2003b).This step ensured that no collinearity was introduced into the analysis. The analytical techniques included linear regression for 2 continuous variables, Pearson χ^2 test or Fisher exact test for 2 categorical variables, and T-test or ANOVA for continuous response in no less than two categories groups. The correlation analysis was conducted between 2 possibly associated predictors based on the causal diagram.

Table 3. 1 Overview of the variables tested in the explanatory data analysis for association with bovine digital dermatitis of 32,742 cows from 124 dairy herds in the North Taranaki, New Zealand.

Variables	Type of variables
1. Numbers of acquired calves from external source	Continuous
2. Source of acquired calves	Categorical
3. Number of acquired dairy cows from external source	Continuous
4. Source of acquired dairy cows	Categorical
5. Number of acquired bulls from external source	Continuous
6. Source of acquired bulls	Categorical
7. Total number of cattle acquired to the farm	Continuous
8. Transportation method	Categorical
9. Whether or not sharing transportation	Categorical
10. Frequency of performing hoof trimming	Categorical
11. External Staff coming to perform hoof trimming	Categorical
12. Percentage of hoof trimming done by external staff	Continuous
13. Feeding system	Categorical
14. Shed type	Categorical
15. Herd Size	Continuous
16. Month of inspecting the farms	Categorical

Multivariable data analysis

The dataset was arranged in an aggregated form. Each row recorded each farm's information, i.e. counts of BDD cases within a farm and farm level exposures. As the visual inspection was performed for every single cow, the disease status of each cow follows Bernoulli distribution. Therefore number of diseased cow within a herd follows binomial distribution. Therefore the aggregated form could also be called binomial form. A clear explanation of the data structure would be helpful to understand three different modelling strategies used to compare. The best-fitted model would be reported as the final model. The three potential models are listed below:

1. A generalised estimating equation (GEE) model (Liang & Zeger, 1986) was fitted with a binomial distribution and logit link using robust standard error to adjust within farm clustering. The working correlation matrix was defined as exchangeable (Chen & Lazar, 2012).

2. A generalised linear model was fitted with Poisson distribution, log link (Gardner, Mulvey, & Shaw, 1995) and setting the ln(herd size) as an offset. Farms are independent, therefore this procedure considered the dataset as one level data (farm level).

3. A generalised linear model was fitted with negative binomial distribution, log link (Gardner et al., 1995; Lawless, 1987) and setting the ln(herd size) as an offset. Same as the Poisson model, the data were one level as farms are independent. The aim of using this model was to deal with overdispersion.

Bivariate analysis of each predictor and outcome (BDD) were conducted to understand the gross association between predictors and outcome. The linearity of continuous variables was assessed by plotting smoothed lines computed from log-odds scale of each continuous variable against each continuous predictor (I. Dohoo, W. Martin, & H. Stryhn, 2003a; I. Dohoo et al., 2003b). If they violated the linearity assumption, they would be categorised and their categorical form would be used in further analysis. For those predictors which had P values less than 0.20 from bivariate analysis would be considered into multivariable model. A backward strategy was used in which the variable had the highest P-value among all variables in the model was deleted. The P values were produced by Wald test (Barnhart & Williamson, 1998; Molenberghs & Verbeke, 2007). The model was refitted until all the remained variables showing $P \leq 0.05$. However, in case a variable was removed from the model and the coefficients of other variables in the model changed around 15% or more, the removed variable would be considered as a confounder and therefore it would be retained in the model. After building the statistically significant model, *a priori* including the variables which were not considered in the multivariable model due to the $P > 0.20$ generated from the bivariate analyses or eliminated during model building stage but biologically important were added back in the model, one at time (I. Dohoo et al., 2003b). Those variables would be removed from the multivariable model if they were not exposures of main interest or they were statistically non-significant. Two-way interactions between all the predictors remained in the preliminary main effect model were created. The statistically significant interaction terms which were also biologically plausible would remain in the model (I. Dohoo et al., 2003b). There were no three-way interactions examined.

The analysis was performed using Stata/IC 13.1. The significance level was set at P=0.05.

Model Diagnosis

Under the condition that those models contained all the biological confounders and interactions, the amount of dispersion of the three mentioned models were evaluated by dividing the Pearson χ^2 by its degree of freedom (DF). For the GEE binomial model, the Pearson residual plots based on the quantile–quantile (Q–Q) plots of χ^2-distribution were the alternative way to look at the goodness-of-fit (Oh, Carriere, & Park, 2008). DFBETAs statistics which are scaled measures of the change in each parameter estimate were used to identify the outliers, the threshold was $2\sqrt{n}$ (Patterson, 1981). For Poisson and negative binomial model, Akaike's information criterion (AIC) (Bozdogan, 1987) was used to compare the goodness-of-fit between them. Anscombe residuals versus predicted BDD positive counts were plotted to identify outliers (I Dohoo, W Martin, & H Stryhn, 2003). The statistical outliers were therefore checked to figure out if they were biological plausible. Only the non-bilogical plausible outliers were deleted and then refitted the model.

3.4 Results

3.4.1 Causal Diagram

Based on researchers' hypothesis, a causal network diagram (Figure 3.1) revealed that prevalence of bovine digital dermatitis (BDD) was directly affected by month; meanwhile month was independent of other covariates. Hoof trimming done by the external staff could affect BDD directly by contacting with the milking cows. However, failing to reach a certain workload might not affect BDD occurrence. Besides, calling external staff to perform hoof trimming might depend on the frequency of performing hoof trimming. In this study, we were interested in the association between BDD and introducing animals which includes numbers of animals introduced, type of animals introduced and source of animals introduced. These mentioned variables could be confounded by the herd size. Herd size could be reflected by different shed, a rotary shed could be normally seen in a big herd, while herringbone shed was commonly in the smaller herd. Housing system was reported as an important risk factor in intensive dairy system in the North Hemisphere. In this study, feeding system which referred to housing system in EU or American studies was also investigated and considered to be confounded by herd size.

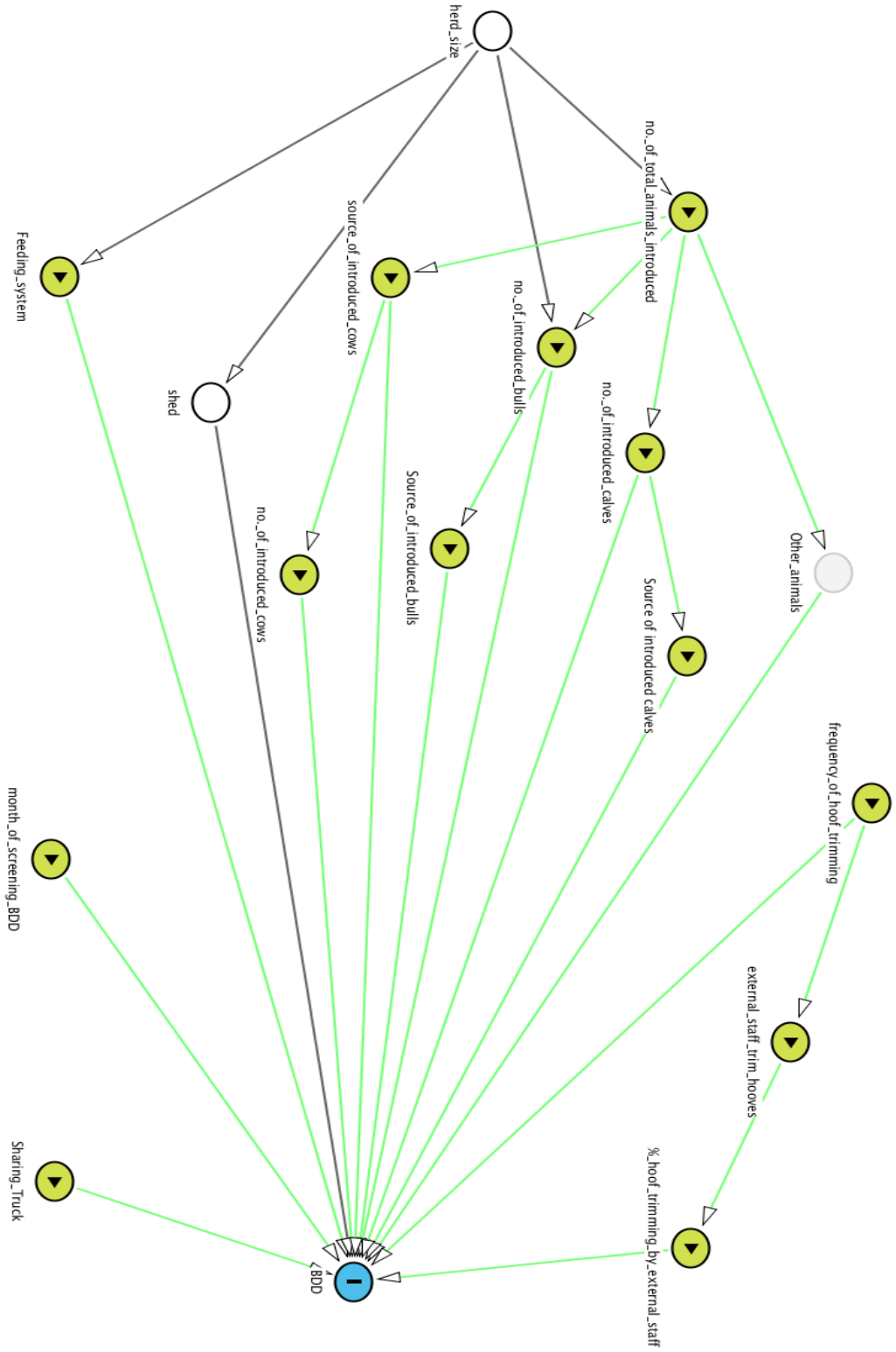

Figure 3. 1 Hypothesised causal model including all variables to identify the potential risk factors, confounders and intervening variables on bovine digital dermatitis in pasture-based system in the North Taranaki, New Zealand from September 2014 to February 2015. White donates potential confounders, grey donates latent variable, yellow donates the exposures and blue donates the outcome.

3.4.2 Explanatory Data Analysis

Questionnaires were received from 124 farms when writing this report. After validating the data, 10 missing values were detected from 5 different variables; they were source of acquired animals (dairy cows (2), calves (1) and bulls (1)), number of introduced bulls (1) and transportation related variables (sharing transportation tools or not (1), transportation method (4). The 10 missing values were found in 10 unique farms.

None of the continuous variables were normally distributed restrictedly. Out of 124 farms, 82 of them reported they did not introduce dairy cattle to their farms by external source; 74 of them reported they did not introduce calves from external source and 41 of them reported they did not introduce bulls over the previous 12 months. After excluding the farms which did not acquire the certain type animals, the median values of acquired dairy cattle, calves and bulls were 18, 43 and 4, respectively. The median herd size and the median total number of introduced animals were 238 and 31, respectively (Table 3.2). The total animals introduced in a farm were consisted of introduced calves, dairy cows, bulls and other type of animals. Only 9 farms reported they did not introduce any animals over the previous 12 months.

Table 3. 2 Central tendency and spread for continuous variables from 124 farms in the North Taranaki, New Zealand from September 2014 to February 2015.

Variable	Minimum	25th Quartile	Median	75th Quartile	Maximum
Number of acquired calves	1	12	42.5	60	300
Number of acquired dairy cows	1	5	17.5	50	510
Number of acquired bulls	1	3	4	6	15
Total number of acquired cattle	0	5	30.5	66.5	533
Herd size	52	177.5	240	320	1155

Hoof trimming by external staff were performed in 88/124 farms. There was only one category of external staff – vets. For the farms which had vets to perform hoof trimming, the workload of the vets varied from 1% to 100%, the median workload was 20%.

For categorical variables, 100% farms trimmed lame cows only; 68% farmers paid transportation companies to transport their animals; only 7 farmers shared trucks with their neighbours. Farmer acquired calves and dairy cows mainly from other farms, while for bulls

both other farms and dealers were common source of requiring. Thirty-three farms had history of receiving grazing-off heifers from other farms. Details of frequency and percentage of categorical variables which would be used for further analysis were summarised in Table 3.3.

The correlation analysis suggested the number of acquired cows, calves and bulls were not correlated with number of total animal acquired, also not correlated to each other. The correlation coefficients between any two variables were less than 0.2. The herd size was significantly different in farms with different milking shed. The average herd size in rotary shed was 328 while the average herd size in herringbone shed was 229 ($p<0.01$). Farms providing feed pads had significantly larger herd size (351) than farms without feed pads (248) ($p<0.01$).

Table 3. 3 One way table to describe the farm level variables which would be used in the multivariable model in 118 farms in the North Taranaki, New Zealand from September 2014 to February 2015.

Variable	Frequency	Percent	Variable	Frequency	Percent
Truck Sharing			Feeding system		
No	111	94.1	Pasture	99	83.9
Yes	7	5.9	Pasture + Feed pad	19	16.1
Total	118	100	Total	118	100
Shed			Hoof trimming by vets		
Herringbone	78	66.1	No	35	29.7
Rotary	40	33.9	Yes	83	70.3
Total	118	100	Total	118	100
Month			Source of acquired calves		
Sep-14	21	17.8	Not acquired	70	59.3
Oct-14	26	22.0	Other farms	37	31.4
Nov-14	18	15.3	Dealers + Markets	11	9.3
Dec-14	22	18.6	Total	118	100
Jan-15	21	17.8	Source of acquired cows		
Feb-15	10	8.5	Not acquired	78	66.1
Total	118	100	Other farms	26	22.0
Number of total animals introduced to the farm			Dealers + Markets	14	11.9
0-5	30	25.4	Total	118	100
6-30	28	23.7	Source of acquired bulls		
31-66	30	25.4	Not acquired	40	33.9
67-533	30	25.4	Other farms	40	33.9
Total	118	100	Dealers + Markets	38	32.2
Receiving grazing-off heifers from other farms			Total	118	100
No	97	78.2			
yes	27	21.8			
Total	124	100			

3.4.3 Risk factors

Obvious overdispersions were found in the GEE binomial model (Pearson χ^2/DF = 3.68) and the Poisson model (Pearson χ^2/DF = 3.98), however overdispersion was not of concern

(Pearson χ^2/DF = 0.93) in the negative binomial model. The AIC of the Poisson model and the negative binomial model were 5.78 and 4.28, respectively. This suggested the generalised linear model with negative binomial distribution would be selected as the final model. The multivariable negative binomial model contained six main effects. No significant interactions were found. The model was presented after deleting the outliers (Table 3.4).

Table 3. 4 Multivariable generalised linear model including risk ratio and 95% confidence interval for the variables associated with bovine digital dermatitis among 30,426 cows on 114 farms in the North Taranaki, New Zealand.

					95% Confidence interval	
Exposure	**Category**	**Count**	**P**	**Risk Ratio**	**Lower**	**Upper**
Shed type	Herringbone	75		Ref		
	Rotary	39	0.075	1.70	0.95	3.05
Months of screening			0.009			
	Sep-14	18		Ref		
	Oct-14	26	0.028	0.41	0.19	0.91
	Nov-14	18	0.171	0.56	0.24	1.29
	Dec-14	22	<0.001	0.19	0.08	0.45
	Jan-15	20	0.018	0.38	0.17	0.85
	Feb-15	10	0.293	0.59	0.22	1.58
Herd size[1]	Continuous		0.276	0.85	0.64	1.14
Purchasing cows in last year	no	79		Ref		
	yes	35	0.058	1.69	0.98	2.91
Receiving grazing-off heifers[2]	no	91		Ref		
	yes	23	0.022	2.06	1.11	3.83
Hoof trimming	farmers	33		Ref		
	vets	81	0.007	2.28	1.26	4.13

1: Herd size was presented as risk ratio per standard deviation (sd=136)
2: Some dairy farms received grazing-off heifers from other farms in the previous 12 months

Compared to herringbone herds, BDD cases were more likely to observe in rotary herds (RR=1.70, 95%CI: 0.95-3.05). Compared to September, less BDD cases was detected in October (RR=0.41, 95%CI: 0.19-0.91), December (RR=0.19, 95%CI: 0.08-0.45) and January (RR=0.38, 95%CI: 0.17-0.85). However, counts of BDD cases in November (RR=0.56, 95%CI: 0.24-1.29) and February (RR=0.59, 95%CI: 0.22-1.58) were not significantly different from September (Figure 3.2). Besides the monthly cow level prevalence, the monthly farm level prevalence was also described (Figure 3.3). Herd size was not significantly associated with BDD (RR=0.85, 95%CI: 0.64-1.14), but it was an important confounder. Compared farms not purchasing cows, farms which brought cows in had higher prevalence of BDD (RR=1.69,

95%CI: 0.98-2.91). Farms which brought grazing-off heifers in from other farms had higher prevalence of BDD (RR=2.06, 95%CI: 1.11-3.83) compared to farms not receiving grazing-off heifers. Farms were at higher risk of having BDD if external staff (i.e. veterinarians) was used to trim feet (RR=2.28, 95%CI: 1.26-4.13), compared to farms where all foot trimming was undertaken only by farm staff.

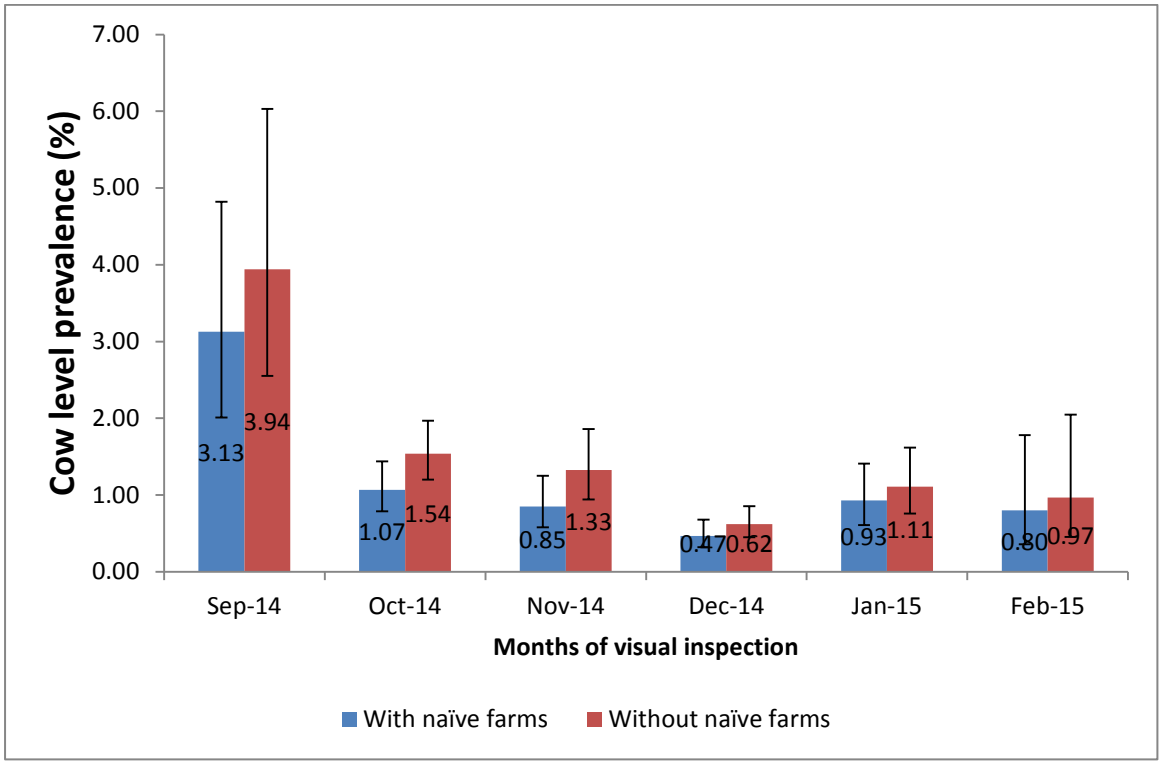

Figure 3. 2 Monthly cow level prevalence of bovine digital dermatitis in 224 farms and 143 affected farms in The North Taranaki, New Zealand from September 2014 to February 2015.

3.5 Discussion

3.5.1 Discussion of the negative binomial model

The objective of the study was to identify farm level risk factors of bovine digital dermatitis (BDD) in pasture-based dairy herds in the North Taranaki. More than 50% farms (124/224) responded and returned questionnaires of which farms could be used in data analysis after data validation. The final model included 114 farms after excluding four outliers. The variance function of the negative binomial model was shown below:

$$V(u) = u + \alpha u^2$$

where α refers to the dispersion parameter and μ is the mean.

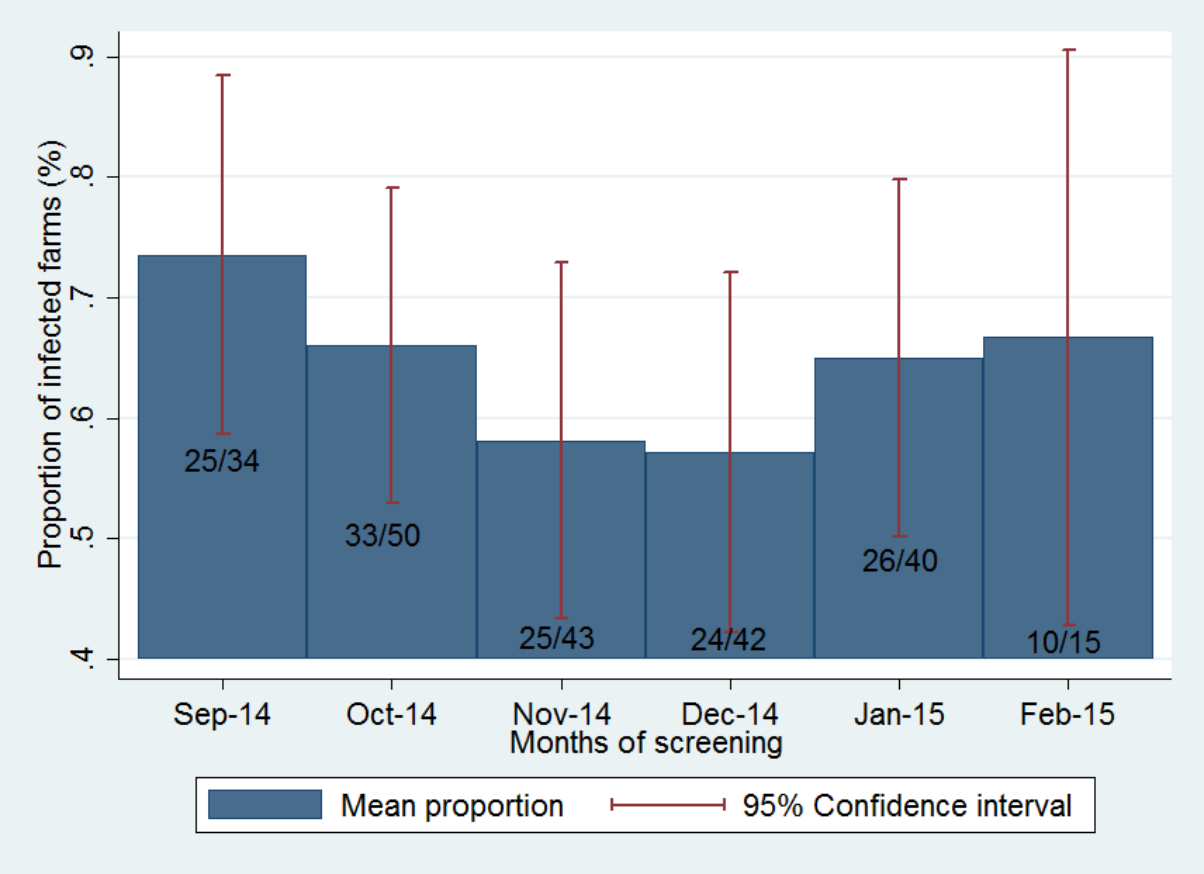

Figure 3. 3 Monthly farm level prevalence (proportion of infected farms) of bovine digital dermatitis in 224 farms in The North Taranaki, New Zealand from September 2014 to February 2015. Values on the bars were expressed as 'number of positive farms/number of farms inspected' in each month.

Setting the negative binomial distribution in the generalised linear model (GLM) requires a known dispersion parameter (α). To obtain α, a negative binomial regression using full maximum likelihood (ML) estimation was required. ML estimation can produce the α which could be set when running the GLM (I Dohoo et al., 2003). The merit of using GLM is that it gives AIC, BIC (Bayesian information criterion) and many GLM-defined residuals to assess the goodness-of-fit and to identify outliers (I Dohoo et al., 2003).

3.5.2 Month of screening

The month of screening reflected both climate related information and cows lactation stages. Higher BDD prevalence in September coincided with early spring and high lactation which meant the climate was still cold and wet. Cows were likely to graze muddy pastures which had been identified as an important risk factor overseas (Frankena et al., 1993; RodriguezLainz, David, et al., 1996). Extensive and long exposure to poor hygiene such as slurry and moist underfoot conditions might soften the hooves and lead to hoof abrasions so that the bacteria from mud or faeces could cause secondary infection.

Our findings suggested that early lactation (30 to 60 days in milk in September) was a high risk period for BDD within the six months covered by this study. It agreed with reports from the Netherlands (K. Frankena et al., 1991; Holzhauer et al., 2006). Holzhauer et al. (2006) suggested that 30 to 60 days in milk interacted with third parity (OR=1.4) suggesting that high producing cows at peak milking had the highest risk. Frankena et al. (1991) again found that cows in 30 to 70 days in milk were more risky to be affected by BDD. However, several other researchers reported the assocation between different lacation stage and BDD differently. A retrospective study in Mexico (ArgaezRodriguez, Hird, deAnda, Read, & RodriguezLainz, 1997) suggested that the first 30 days in milk, i.e. transition and immediate post-calving periods, posed the highest risk of cows being affected by BDD. In our study, the screening had not started in the first 30 days in milk. Therefore, the association between first 30 days in milk and BDD could not be evaluated. In addition, a research in Chile by screening method reported that the odds of BDD were increasing with an increasing days in milk (Rodriguez-Lainz et al., 1999). Moreover, these authors concluded that they could easily diagnose the lesions in later lactation when they were larger, while the difficulty of diagnosing lesions in our study was not altered in different months.

A few overseas studies also compared difference of the risk of BDD in dry-off period and lactating period (ArgaezRodriguez et al., 1997; Murray, Downham, Demirkan, & Carter, 2002; Somers et al., 2005a). The conclusions from all the mentioned studies were consistent that the drying-off cows were less infected with BDD than the lactating cows.

3.5.3 Shed type and herd size

Our hypothesis was that the sensitivity of screening BDD was altered in different milking shed (Rodriguez-Lainz et al., 1999). During the screening, we found it was easier to find BDD cases in rotary shed compared to herringbone shed because enough time were allowed to wash the cows' rear feet carefully. Shed type might reflect the other farm characteristic – herd size. In correlation analysis, the T test suggested that the rotary herd had significantly more cows than the herringbone herd. Therefore, the herd size was adjusted in the model to compare the BDD prevalence in different shed type given the fixed herd size. The marginally significant difference between shed types (p=0.075) suggested that fewer BDD cases were found in herringbone herds, confirmed our biological hypothesis. Overseas

studies suggested that BDD was more likely to transmit within a larger herd (Holzhauer et al., 2006; Rodriguez-Lainz et al., 1999; Wells et al., 1999) because large herd had higher exposure level of pathogens. However, our model suggested BDD cases were not associated with the herd size (p=0.276), which could be explained that the contacts between cows were less in pasture-based dairy farming system compared to the housing system.

3.5.4 Introducing animals into the farms

Whereas a Dutch study suggested that buying dairy cattle (binary variable) in the last year had no effect on BDD (Somers et al., 2005a), we found that farms purchasing dairy cattle had higher risk of BDD infection than closed farms (RR=1.69). This could be explained with more frequent contacts with other animals and hence with BDD causing pathogens from external sources.

Besides the effect of purchasing dairy cattle, receiving grazing-off heifers from other farms also made the farms at risk of BDD (RR=2.06). This suggested that heifers in the North Taranaki might be infected by BDD already. This finding was consistent with a Mexican study. That study suggested that heifer-buying farms had higher risk of BDD compared to no heifer-buying farms when the cows on farm were exposed to the muddy environment (RodriguezLainz, David, et al., 1996).

Although introducing milking cows and grazing-off heifers are identified as risk factors, introducing bulls was not significantly associated with BDD (therefore excluded from the model). This could be explained by the small number of bulls purchased by dairy farms, generally during the late mating period.

3.5.5 Feeding system

As many studies in the northern hemisphere suggested that keeping cows indoor was an important risk factor (Capion, 2004; R. A. Laven, 1999; Rodriguez-Lainz et al., 1999; Somers et al., 2003), we considered in the pasture-based dairy system, crowing cows on feed pads before milking might a proxy for a higher density as in indoor systems. However, this was not a significant predictor in our study (therefore excluded from the model). Perhaps the time that cows spent on feed pads was too short to exert an effect on BDD.

3.5.6 Hoof trimming

Hoof trimming was considered as an important risk factor investigated by many studies. Three factors may be important, (i) the frequency of hoof trimming and its effect on claw health, (ii) hoof trimming as a means of transmitting BDD causing pathogens, and (iii) the number of staff involved in hoof trimming of a farm.

The effect of hoof trimming frequency on BDD could not be evaluated in this study because all farmers trimmed only lame cows. Holzhauer et al. (2006) reported a negative association between long hoof trimming interval (>12 months) and BDD; whereas Rodrigues-Lainz et al. (1999) and Somers et al. (2005) reported the opposite effect, i.e. that long hoof trimming interval increased the risk of infection with BDD. Holzhauer et al. (2006) argued that failing to clean equipment between cows and farms could promote pathogen transmission. In our study, cows were at higher risk for BDD if their hooves were trimmed by vets (external staff) than trimmed by farmers (internal staff), suggesting that external staff and their equipment might be likely to act as fomite for transmission, possibly exacerbated by the higher workload of vets. The statistic model however, suggested that BDD infection was not significantly associated with the workload of hoof trimming by vets. The model only suggested that hoof trimming by external staff (i.e. vets), was a risk factor for cows to be infected with BDD.

3.5.7 Outliers

The 4 outliers excluded from the analysis had the same feature. More than 90% of cows in the milking herds were purchased in previous 12 months. Among those 4 farms, 3 farms reported the number of puchased cows in last 12 months were larger than the herd size on the day of screening. It could be plausible that they purchased a lot of cows at the beginning, due to many reasons, they culled some cows, the remained milking herds were smaller than the number of purchased cows at the beginning. This farm management might be seen in new established farms, but this management did not present the common dairy farm management in the North Taranaki. Since they were both statistical and biological outliers, they were excluded in the final model.

One limitation of our study was that diagonosing BDD purely replied upon visual assement could miss many cases as mentioned in the previous chapter. In addition, screening for the

whole population rather than calculating the required sample size made collecting information of exposures difficult. Therefore, no cow level risk factors such as breed, age, or even more precise lactation stage were investigated in the study. Apart from those two shortages, recall bias might be occurred as farmers were not required to respond questionnares on the day of inspecting their cows. A great many of them answered the questionnaires long time after the inspection.

3.6 Conclusion

This study identified some management-related and biosecurity-related risks of BDD in the North Taranaki. Over the study duration from September 2014 to February 2015, the BDD prevalence was highest in September and lowest in December. However, this finding might be confounded by lactation stage and explained by high lactation as in other studies. Introducing milking cows and grazing-off heifers into the herd were risk factors for BDD. However, introducing bulls for breeding did not increase the risk of BDD. Hoof trimming performed by vets posed a higher risk than trimming by farmers themselves, which may be related to the disinfection of trimming equipment.

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