Epidemiology of BVD in New Zealand dairy herds

A thesis presented in partial fulfilment of the
requirements for the degree of

Doctor of Philosophy

In

Veterinary epidemiology

at Massey University, Manawatu, New Zealand.

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2016

Abstract

The objective of this thesis was to determine the prevalence and incidence of bovine viral diarrhoea (BVD) virus at cow and herd level, define risk factors for new infection and to quantify the impact at cow level of seroconversion during the seasonal breeding programme under the seasonal calving, pasture grazed systems in New Zealand.

A questionnaire and bulk tank milk (BTM) BVD PCR and antibody ELISA test was completed for 402 New Zealand dairy herds, and repeated in the subsequent lactation. North Island herds had a high turnover rate with 67% of virus positive herds clearing infection each lactation and being replaced with newly infected herds, while the larger South Island herds rarely cleared infection naturally (14% per lactation) and maintained a higher prevalence (32% compared to 8.5% for North Island herds). Transmission pathways associated with bulk tank BVD status were purchasing cows, neighbour's stock, and stock movements off-farm. The other factors associated with bulk tank BVD status were herd size, herd BVD vaccination, and herd ownership structure.

In 10 BTM PCR positive herds, all lactating cows (n=3,793) were tested for BVD antibody at the start of the seasonal breeding programme (planned start of mating; PSM), and again 125 days later, to identify cows that seroconverted during the observation period. Improved cut-off values were derived for the IDEXX milk antibody ELISA. There were few (3.8%) susceptible lactating cows at PSM in herds with a lactating persistently infected cow (PI), but most of these susceptible cows (82%) seroconverted. This required 4.6 contacts per PI each day. There were more susceptible (31%), and a smaller proportion of susceptible cows seroconverted (32%) in herds without a lactating PI. Seroconversion was associated with 13% longer PSM to conception (3.2 days), 4% lower pregnancy rate, 6% lower conception to AI, and $11.97 (1.9 times) greater cost of clinical disease. The average cost per transient infection was $91.08.

These results contributed to voluntary BVD control efforts in New Zealand and will be essential for developing a comprehensive cost-benefit model to estimate the average total cost of BVD, and assessing the benefit of various control strategies.

Keywords: Bovine Viral Diarrhoea; BVD; BVDV; virus; diarrhea; pestivirus; Flaviviridae; veterinary; epidemiology; New Zealand; dairy; prevalence; incidence; herd; cow; reproduction; disease; transient infection; immune suppression; PI; PCR; ELISA; antibody; milk; economic; cost; seasonal; pasture-based; observational study; longitudinal; cross-section; risk factor; risk; probability; proportion; rate; mastitis; lactation; seroconversion; regression; generalised estimating equation; GEE; Hurdle model; accelerated failure time; AFT; questionnaire; survey; sharemilker; cow behaviour; herd management; model.

Acknowledgements

I would like to thank the farmers involved for their time, completing and returning questionnaires, giving permission to test their bulk milk, the use of their facilities and animals, and for following instructions and recording activities and events; New Zealand dairy farmers for funding the study through DairyNZ; The McKenzie legacy trust for additional funding contributions; Eltham District Veterinary Services for financial support, in-kind contributions (particularly Joan Hughes), and the patience and encouragement from other staff members; Livestock Improvement Corporation for in-kind contributions toward the testing costs, particularly Hinrich Voges who provided valuable feedback and advice about diagnostic testing; my supervisors Cord Heuer, Scott McDougall, and Mark Stevenson for their guidance, advice, and feedback. I would also like to acknowledge my family: my wife Kath and my children Sarah, Esther, and Daniel who sacrificed much for this PhD, and my parents Alistair and Heather Weir for their on-going support.

Approval for this research was obtained from the Massey University Animal Ethics Committee.

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Chapter 1: Introduction

Bovine viral diarrhoea (BVD) virus afflicts cattle everywhere except where it has been eradicated (Lindberg and Alenius 1999). The prevalence of BVD virus seropositive animals in the general dairy population is usually 60-85% (Houe 1995, 1999) with a variable prevalence of virus positive herds from <1% to >50% (Rufenacht *et al.* 2000; Joly *et al.* 2005; Kozasa *et al.* 2005; Houe *et al.* 2006). Exposure of naïve animals to BVD virus shortly before or during the first third of pregnancy can lead to failure to conceive, pregnancy loss, mummification, persistently infected (PI) animals (that often do poorly and can subsequently die from mucosal disease) and numerous developmental defects, the outcome being dependent upon the stage of gestation when infection occurs (Done *et al.* 1980; McGowan *et al.* 1993a; Moennig and Liess 1995). The economic cost of BVD can vary greatly from herd to herd depending on the herd size, initial herd immunity, strain of infecting virus and stage of the breeding cycle when infection occurs, but at a whole population level losses have been estimated at US$10-57 per calving (Spedding *et al.* 1987; Houe *et al.* 1993b, 1993a; Houe 1999).

Maintenance of infection usually requires the presence of a PI animal (Lindberg and Houe 2005). These are most often created when a naïve cow is infected between 18 and 125 days of gestation (Grooms 2006) resulting in the persistent infection of the immunotolerant calf (Brownlie *et al.* 1984). However, vertical transmission from a PI cow to her offspring is thought to be assured (Brownlie *et al.* 2000). The resulting PI animal remains immunotolerant throughout their lifetime and continuously sheds virus (Brownlie *et al.* 1987; Brock *et al.* 1991). They can infect more than 90% of animals in a few months given close contact in relatively small groups (Houe *et al.* 1993b; McGowan *et al.* 1993a; Moerman *et al.* 1993). Individual animal testing for virus is necessary to identify persistently infected animals and it can be time-consuming and expensive, especially in large herds. An initial estimation of BVD virus infection status of the herd can be made using a bulk milk sample (Waage *et al.* 1994; Bitsch and Ronsholt 1995). There is good correlation between seroprevalence and bulk tank antibody levels (Niskanen 1993). There is also a strong association between seroprevalence and the likelihood of a PI being present (Houe and Meyling 1991). Considering the long duration of immunity (seropositive status) in previously infected individuals (Duffell and Harkness 1985; Fredriksen *et al.* 1999) current active infection in the milking herd (presence of a milking PI) cannot be distinguished from recently cleared infection by antibody testing (Houe 1994).

Dubovi (1994), and Grooms (2006) have reviewed the effect of BVD virus infection on reproductive function. Susceptible animals infected around the time of mating can experience reduced conception rates (McClurkin *et al.* 1979; Whitmore *et al.* 1981; Houe *et al.* 1993b; McGowan *et al.* 1993a; McGowan *et al.* 1993b). Infection from around 18 days after mating can result in embryo loss (McGowan *et al.* 1993a). Herds with active infection can experience an increased rate of abortion (Barber *et al.* 1985; Roeder *et al.* 1986; Sprecher *et al.* 1991; Larsson *et al.* 1994), and this effect has been demonstrated by experimental infection (Casaro *et al.* 1971; Done *et al.* 1980). The association between BVD status and reproductive performance has been investigated at the individual animal level using serum antibody status (Houe *et al.* 1993b; Larsson *et al.* 1994; Rufenacht *et al.* 2001), and at the herd level using two or more bulk tank milk samples at intervals of 4-18 months to determine the herd BVD status (Niskanen *et al.* 1995; Fredriksen *et al.* 1998; Valle *et al.* 2001; Robert *et al.* 2004). Study results for the effect of BVD virus infection on reproductive performance have been inconsistent. Differences in study design, power, classification of BVD status and method of analysis would have contributed to this variability. Only two studies accounted for clustering of cows within herds (Rufenacht *et al.* 2001; Valle *et al.* 2001), while others only adjusted for some herd effects as fixed effects in their models (Niskanen *et al.* 1995; Robert *et al.* 2004).

Estimates of the association between BVD infection and cow health and production have also been inconsistent. A sudden reduction in milk yield has been described in case studies of individual herds experiencing a BVD outbreak (Barber *et al.* 1985; Larsson *et al.* 1994). Moerman *et al.* (1994) found a significant reduction in milk yield in seroconverting cows compared to herd mates that did not seroconvert. The number of transiently infected animals, and the magnitude of the decrease may make it difficult to demonstrate the effect in large-scale studies with a less precise risk period (Niskanen *et al.* 1995). Due to the immunosuppressive effects of BVD (Reggiardo and Kaeberle 1981; Roth *et al.* 1981; Bolin *et al.* 1985; Kovacs *et al.* 2003) and the impact of impaired neutrophil function (Cai *et al.* 1994) an increase in concurrent disease conditions might be expected in virus positive herds. An increase in clinical mastitis incidence has been associated with an increasing or persistently high bulk tank BVD antibody level (Niskanen *et al.* 1995; Waage 2000). Increased risk of retained fetal membranes (RFM) was recorded by Larsson *et al.* (1994) in one BVD affected herd, but Fredriksen *et al.* (1998) failed to identify an increased risk of RFM or metritis in 32 BVD virus infected herds in Norway.

Transient infection in calves has been associated with a number of consequences. When concurrently infected with BVD virus, calves infected with other pathogens have more severe clinical signs (Castrucci *et al.* 1992; Ganheim *et al.* 2003), as well as more severe leukopenia (Ganheim *et al.* 2005) and greater viral shedding in nasal secretions for longer duration (Brodersen and Kelling 1998). BVD virus causes villus atrophy and submucosal inflammation and potentiates these effects with concurrent Rotavirus infection (Kelling *et al.* 2002). The antibody response to other pathogens is delayed with lower final titres with concurrent BVD virus infection (Elvander *et al.* 1998). Calf disease prevalence and mortality rate is increased when BVD virus is present (Larsson *et al.* 1994).

Most New Zealand dairy herds have highly seasonal calving, pasture-based management systems (Verkerk 2003), and larger herds than many other regions which may have a substantial impact on the epidemiology of BVD virus. While the first confirmed isolation of BVD virus was made in NZ in 1967 (Jolly *et al.* 1967) a previous serosurvey of 118 cows for respiratory disease showed 35% had antibodies to BVD virus (Fastier and Hansen 1966). Subsequent surveys in both beef and dairy cattle have recorded prevalence rates of 34-60% (Durham and Forbes-Faulkner 1975; Littlejohns and Horner 1990; Perez *et al.* 1994; Perez *et al.* 1995). More recently, bulk tank ELISA antibody tests on 724 herds in the North Island found that 90% of herds had greater than 30% within-herd prevalence (Thobokwe *et al.* 2004), using the classification of Beaudeau *et al.* (2001a). Another study found 93% to 97% of herds from one practice had BVD antibodies in the bulk tank, depending on the test used (Compton and McDougall 2005). New Zealand's extensive grazing systems may be associated with a lower seroconversion rate than often seen in small, housed herds such as Houe et al. (1993b). For example, Thompson (2005) found only 78% of 133 heifers had seroconverted over nearly 2 years despite continuous mixing with 32 PI animals. A New Zealand study of PI animals (Voges *et al.* 2006) found that they had an average 18% slower growth, 50% lower production, 6% higher abortion rate, 17.2 times higher odds of culling for mastitis related reasons, and 12.6 times higher odds of loss due to severe illness or sudden death, and only 4% of the odds of surviving to the end of the study at 4.5 years old despite no culling for production associated reasons, although 75% of the PI animals survived to enter the lactating herd which was much higher than expected (Houe 1993). Estimates of economic loss due to BVD were around $87 per cow per year in herds with high levels of antibodies compared to herds with lower antibody levels and $44.5 million per year for the New Zealand dairy industry (Heuer *et al.* 2007).

Before 2007 when this project began, only a small proportion of New Zealand dairy herd managers understood BVD, and there were many conflicting opinions about what should or could be done about

BVD even among veterinarians (Thompson 2005). There was some interest in BVD control among dairy veterinarians, but no consensus about whether control was worthwhile. A voluntary BVD steering committee was formed in 2005 to examine the feasibility of controlling BVD, following a BVD symposium (Lindberg 2005). The steering committee concluded that "a formal control programme was not feasible at [that] time due to limitations in diagnostic techniques, industry awareness, and economic impact data", and division in the veterinary profession[1]. Most New Zealand veterinarians were not confident that they understood the sources and associated risks of virus introduction, or the efficacy or cost-benefit of control options. Most veterinarians agreed that bull testing was likely to be worthwhile, and some recommended BVD vaccination in some situations. Most of the epidemiological information about BVD came from year-round calving, partial or full housing systems and often with much smaller herd size than was common in New Zealand. The economics of these systems was also different than New Zealand's pasture-based system with low production per cow and no government financial support or production control systems. There was little confidence that these epidemiological or economic studies applied to the New Zealand industry.

In summary, key aspects of the epidemiology of BVD were well established such as the creation and role of PI animals. Other aspects such as the reproductive effects have been demonstrated numerous times but the range of effect sizes and which effects were observed varied from study to study. This may have been due to viral strain and biotype differences, as well as herd management, cow-factors, and study design differences (Houe 1999), so New Zealand observational research with local strains was highly desirable. The immunosuppressive effects of BVD in calves are well-established, and New Zealand neonatal calf rearing practices are more similar to European systems than lactating cow management practices so are likely to be more directly comparable. Although potential mechanisms have been demonstrated, the available data for the impact of immunosuppression in lactating cows was more contradictory and sparser than for calves and was restricted to herd-level incidence rates.

There was some limited New Zealand prevalence data and an initial crude estimate of the cost of BVD in New Zealand but what was needed was a comprehensive analysis to integrate the epidemiological information about BVD, and apply it to New Zealand production systems, and to estimate the cost-benefit of various BVD control options. As a precondition however, further research was required to quantify the epidemiological patterns and effects of BVD in New Zealand. Firstly, the infection dynamics for New Zealand seasonal calving, pasture-based herds including the prevalence, the new infection rate, and the natural clearance rate were unknown. These would influence control decisions and be important for designing, tuning, and validating BVD disease models. The bulk tank BVD PCR was a new tool in New Zealand so a comparison between it, and the more established bulk tank antibody ELISA was an additional benefit of this study. Secondly, the relative importance of risk factors associated with herd infection status was of interest. Understanding infection sources and transmission pathways would also be important for planning control measures, and for designing, tuning, and validating BVD models. Finally, it was desirable to have more information about cow-level infection dynamics (prevalence of immunity and incidence of seroconversion), and local estimates of the effects of transient infection in seasonal calving herds including the reproductive effects and cow-level estimates of the effects of immune suppression in lactating dairy cows. In the process of conducting this research, it was discovered that the recommended cut-off values for the antibody ELISA on milk samples were not correct. Thus, further investigation was required to develop optimised cut-off values.

[1] http://www.controlbvd.org.nz/

The aims of the thesis therefore were:

1. To quantify infection dynamics for New Zealand seasonal calving, pasture-based herds including the prevalence, the new infection rate, and the natural clearance rate, and compare the bulk tank PCR and antibody ELISA.
2. To quantify the relative importance of risk factors associated with herd infection status (PCR and antibody ELISA).
3. To optimise cut off values for individual antibody ELISA testing on milk samples.
4. To assess cow-level infection dynamics (prevalence of immunity and incidence of seroconversion), and provide local estimates of the effects of transient infection in seasonal calving herds including reproductive effects and cow-level estimates of the effects of immune suppression in lactating dairy cows.

Chapter 2: Prevalence and changes in bulk tank Bovine Viral Diarrhoea (BVD) virus status in a sample of New Zealand dairy herds.

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Statement of contribution: Appendix 2

Introduction

Bovine Viral Diarrhoea (BVD) virus type I is an encapsulated pestivirus of the Flaviviridae family and is considered one of the most important viral diseases of cattle in New Zealand (Ellison *et al.* 2011). BVD virus is spread and maintained in a population primarily by persistently infected (PI) animals (Lindberg and Houe 2005). Persistent infection occurs when a fetus is infected between 18 and 125 days of gestation (Grooms 2006). Fetuses infected during this susceptible period become immunotolerant and calves, when born alive, shed large amounts of virus throughout their lives. PI animals are therefore the main source of infection and the primary target of disease control.

New Zealand dairy herds have a long history of exposure to BVD virus. BVD virus was confirmed present in New Zealand by virus isolation in 1967 (Jolly *et al.* 1967). An earlier study (Fastier and Hansen 1966) found 9 out of 10 dairy herds had antibodies against BVD virus with an overall cow prevalence of 44%. Of 922 laboratory submissions, 34% were positive for BVD antibodies in 1975 (Durham and Forbes-Faulkner 1975). A stratified random sampling of 784 North Island herds (Thobokwe *et al.* 2004) found 95% of herds had antibodies to BVD virus, and 55% had "high" antibody titres, 32% of a subset of herds were classified as currently infected based on calf antibody testing, and 15% of herds probably had milking PIs based on very high antibody level in the bulk tank. Another study (Compton and McDougall 2005) found 93% to 97% (depending on the test) of 206 convenience sampled Waikato herds had at least some cows with BVD antibodies. A stratified random sample of 350 herds from all regions of New Zealand (Voges 2008) compiled after the current study began found 7% of herds had no exposure to BVD while 35% had recent active infection varying from 11% to 57% by region (11% in Taranaki, 36% in Greater Waikato, and 56% in Canterbury). There is no data on the incidence of the introduction of BVD virus into dairy herds in New Zealand. The prevalence of herds with immune cows most often ranges from 70% to 100% but has been reported as low as <1% (Houe 1999). The prevalence of herds with PI cattle ranges from 53% (Houe and Meyling 1991) to 15% (Houe *et al.* 1995b).

New Zealand dairy farms have differences from many other dairy production systems worldwide that contribute to uncertainties about the infection dynamics of BVD virus transmission between cows and herds in New Zealand. Over 95% of dairy farms are strictly seasonal (Verkerk 2003) so cows that fail to conceive during a fixed mating period are usually culled at the end of the season. It is common practice to graze groups of replacement heifers away from the home farm. Cows are dried off in mobs or all at once. Most feed is pasture or pasture products. The "share milking" system impacts on herd dynamics and stock movements. Herd managers known as share milkers do not own the land and the proportion of the milk payment they get depends primarily on the proportion of the cows they own, up

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to 50% of the milk value where they own all the cows. Large herds with relatively low per-cow production and long productive lives predominate. Many New Zealand dairy herds use bulls for natural breeding for a limited period after an initial 4 to 6 weeks of artificial insemination. Replacement stock are born to artificially inseminated cows. They are in the critical early pregnancy stage (when BVD virus infection gives rise to a PI-fetus) at the time that breeding bulls are introduced to the herd. The authors are not aware of previously published reports about the infection dynamics in New Zealand.

Understanding the prevalence of herd level infection, and especially the risk of new herd infection and clearance of infection are important parameters for assessing the risks and predicting the effectiveness and benefit of investment in BVD control. This study was therefore conducted primarily to assess the prevalence and annual incidence of new infection and clearance of BVD infection in New Zealand dairy herds and their implications for control. The data collected also allowed investigation of the rate of change of the antibody level in New Zealand dairy herds, and a comparison of antibody ELISA and PCR defined bulk tank status.

Materials and methods

A cross-sectional, and a prospective, observational cohort study were conducted with herd and herd-season as the units of interest.

Herds

A convenience sample of 206 dairy herds out of 276 serviced by one veterinary business (Eltham District Veterinary Services) in the Taranaki region (approximately -39.36 to -39.48 latitude, and 174.08 to 174.40 longitude) of New Zealand were recruited. Bulk tank milk samples were tested for virus by PCR and antibody by ELISA in September and October (Spring in New Zealand) in the 2007-2008 production season. The majority of these herds (194, 94%) were tested again 12 months later. The study herds had a relatively low prevalence of infection (13/206 herds were PCR positive in the 2007-2008 season). To increase the precision of prevalence and incidence estimates further herds were enrolled and sampled in the 2009-2010 and 2010-2011 seasons around Eltham, Morrinsville, Hamilton, Ashburton, and Oamaru. These herds (n=133) were enrolled in the National herd fertility study (Brownlie *et al.* 2011) along with a further 63 herds from a Waikato vet practice (AnexaFVC). The 2007 and 2009 data were classified as season 1, and the 2008 and 2010 data were classified as season 2 for analysis. The number of enrolled herds by season and district is summarised in Table 1.

None of the herds had conducted BVD testing before enrolment and results from the first season were not reported to vets or herd managers until after the testing was completed in the second season. Hence, initial results could not have affected farm management practices or disease control decisions associated with BVD. Herds were not prevented from controlling BVD and the herds already doing some form of BVD control continued after enrolment. Of those herd seasons with information available, 72% tested bulls for BVD virus, 16% vaccinated bulls with a BVD killed vaccine, 8% vaccinated cows, and 15% of the 44% that bought cows tested at least some of them.

There were 25 herds in the Eltham district enrolled in 2007/2008 and 2008/2009, then again as part of the national herd fertility study in 2009/2010 and 2010/2011, providing a total of 4 herd-seasons each. The test results were reported after testing in 2008. Of these herds, 4 were bulk milk virus positive following polymerase chain reaction (PCR) testing in 2008 and cleared naturally without intervention. None of these herds changed control activities during the study. The new infection rate and clearance rate did not appear to change (2, 4, 2, and 2 PCR positive herds from 2007/2008 to 2010/2011, respectively, with 1 maintaining infection between 2007 and 2008, and one herd from 2008 clearing

infection in 2009 then PCR positive again in 2010) after their BVD status was revealed in 2008. Therefore, it is unlikely that the limited information provided in 2008 had a substantial influence on the risk of new infection or clearance in the herds that continued into the 2009-2010 and 2010-2011 seasons, so they were included in the analysis.

Table 1: Enrolled herds by season and district

	North Island			South Island	
Season	Eltham	Morrinsville	Hamilton	Ashburton	Oamaru
2007/08[1]	206	0	0	0	0
2008/09[2]	194	0	0	0	0
2009/10[1]	33*	63	35*	33*	32*
2010/11[2]	32*	62	32*	33*	32*

[1] First season of pair; [2] second season of pair for assessing status change
* National herd fertility study herds (Brownlie et al. 2011), other herds drawn from respondents to a mail-out questionnaire.

Testing

Bulk tank milk (BTM) samples were collected twice in each season, approximately two weeks apart (median 14d, 95 percentile range 12d – 28d). The first sample was scheduled for collection before the start of the seasonal breeding program ('planned start of mating'; "PSM") and at the date when the farmer estimated that "most adult and all of the first calving cows would have calved and entered supply". The median actual initial collection date was 4 days before the PSM (95 percentile range: 26 days before to 18 days after PSM). Samples were tested for presence of BVD virus by real-time PCR with a commercially available PCR kit (VetMAX™ - Gold BVDV PI Detection Kit, Thermo Fisher Scientific, Waltham, MA USA) following RNA extraction with a commercially available kit (MagMAX™-96 Viral RNA Isolation Kit for RNA extraction, Thermo Fisher Scientific, Waltham, MA USA). A commercial ELISA was used to test for the presence of BVD antibody (The IDEXX BVDV Total Ab ELISA Test, IDEXX laboratories, Westbrook, Me, USA) using the overnight incubation protocol. All testing was conducted by a commercial laboratory (Genemark Animal Health, Hamilton, New Zealand). Any signal up to 40 cycles was considered positive for the PCR and if either sample was PCR positive, the herd was classified as virus positive for the season. The ELISA S/P ratio (sample to positive ratio) was interpreted as being equivalent to the concentration of antibody in BTM. The higher of the ELISA results from the two sampling times within season was used as the herd antibody level for the season. The antibody level was categorised as none (S/P ratio <0.25), low (0.25 - <0.50), medium (0.50 - <0.75), high (0.75 - <1.0), very high (≥1.0).

Analysis

Data were entered and stored in a relational database (Microsoft Access), validated using routine procedures, and analysed using R 3.1.2 (R Core Team 2014). Prevalence of herd infection, incidence or clearance of infection, antibody level and bulk tank antibody level change between seasons, and the relationship between status measures (PCR and antibody ELISA) were the outcomes of interest in this study. Due to differences in farm size and management systems, the prevalence and the incidence of new infection or clearance were analysed and reported separately for the North and South Islands.

Prevalence of herd infection

Prevalence was defined as the proportion of bulk tank milk PCR positive herds over the number of herds sampled. The counts of PCR positive & negative herds in the first and second season by island were presented in tables and prevalences calculated with 95% confidence intervals using the exact binomial-based method (Clopper and Pearson 1934). The McNemar test was used to test whether

prevalence changed between the first and second season for North Island herds, South Island herds, and for all herds.

Incidence of new infection or clearance

The incidence of new infection was defined as the proportion of initially bulk tank PCR negative herds that changed to PCR positive in the second season. The incidence of clearance was defined as the proportion of initially bulk tank PCR positive herds that changed to PCR negative in the second season. Confidence intervals were calculated using the exact binomial-based method (Clopper and Pearson 1934). Fisher's exact tests were used to test whether the new infection rate among initially virus negative herds or the clearance rate among initially virus positive herds differed between islands.

The risk of a new BVD infection was defined as the time required for half of the BVD virus negative herds to have experienced BVD infection at some point (not implying a changing prevalence). It was calculated assuming a continuous and even risk across herds and solving: proportion remaining susceptible = $(1 - \text{annual new infection rate})^{\text{number of years}}$ for the number of years. This gave number of years = ln(proportion remaining susceptible) / ln(1 – new infection rate).

Some initially virus positive herds where all PIs died out or were culled during the first season would have maintained a virus-positive status if a new PI was introduced into the herd before sampling in the second season. Because these herds would continue to test positive they would not be classified as cleared. The observed clearance rate (based on herds that cleared and maintained a virus negative status) would, therefore, have underestimated the actual clearance rate. The actual clearance rate may be considered as the clearance achieved if the reintroduction of PI animals was prevented by use of effective BVD control measures. An estimate of the rate of introduction of PI animals can be obtained from the observed introduction rate in herds that initially tested virus negative. The expected total clearance rate assuming effective prevention of reintroduction can be estimated as observed clearance rate / (1 - introduction rate).

The time it would take for North Island and South Island herds to naturally clear infection if reintroduction was prevented was then estimated assuming a fixed annual maintenance rate of 1 – natural clearance rate across all herds. The proportion clearing infection was therefore estimated as 1 – ((1 – the island's annual clearance rate) ^ years) and the number of years required for at least 99% of initially virus positive herds to clear infection was reported.

Level of BVD virus exposure

The antibody categories in each season were presented in a table, the antibody level was sorted and plotted to show the distribution of bulk tank antibody level, and the change between seasons was plotted with PCR status change. The median change in antibody level (S/P ratio in year 2 minus S/P ratio in year 1) was calculated for all herds and an estimate of the median rate of change for herds without evidence of new exposure was estimated by calculating the median for herds where the antibody level was greater than "none", and the antibody level decreased between seasons. The 95% confidence interval for these estimates of median antibody S/P ratio change were calculated using the bootstrap (10,000) resampling method. The potential effect of island was tested using a Wilcoxon rank sum test, for the subset of herds with greater than "none" antibodies and no evidence of new exposure, and the p-value reported. The number and proportion of initially PCR negative herds with evidence of exposure but no virus in the bulk tank milk were reported. Evidence of exposure was defined as either an increase in antibody S/P ratio of 0.1 units or more or remaining in the very high category with no decrease in antibody S/P ratio. The rationale for the second condition was that herds

in the very high category may not have been able to increase further despite exposure, and if they didn't decrease, then the replacement cows were likely to have been exposed. The utility of antibody change for predicting new infection was tested using the receiver operating characteristic area under the curve statistic.

Relationship between bulk tank PCR and antibody level

Bulk tank PCR status was shown on a plot of bulk tank antibody with North and South Island herds combined to illustrate the relationship between these measures. The number and percentage of PCR positive herds in each antibody category were also reported.

The relative risk with 95% confidence interval and the risk difference for high antibody (>0.75 S/P ratio) PCR negative herds changing to PCR positive between seasons was calculated from the 2 by 2 table.

A new infected status was defined as bulk tank antibody level >= 1.0 and the prevalence and status change between seasons based on this variable were compared to the prevalence and status change between seasons based on PCR status using a table of status change categories.

Results

A total of 787 sets of bulk tank results were recorded. Antibodies were present in 41 (95%) herd-seasons (S/P ratio at least 0.2), 175 (22%) herd-seasons had very high antibodies (S/P ratio at least 1.0), and 98 (12%) herd-seasons were PCR positive.

Prevalence of herd infection

A total of 385 herds had 2 seasons of data. The proportion of herds with changing bulk tank BVD PCR status varied markedly by district (Table 2, Table 3). The prevalence of infection was 7.5% (95% CI 4.9% to 11.0%) and 9.7% (95% CI 6.7% to 13.5%) in the first and second season, respectively in North Island herds (8.6% across both seasons, 95% CI 6.5% to 11.0%), and 32% (95% CI 21.2% to 45.1%) and 31% (95% CI 20.0% to 40.3%) in the first and second season, respectively in South Island herds (32% across both seasons, 95% CI 23.7% to 40.3%). There was no evidence that the prevalence changed between the first and second seasons in this data set (McNemar p=0.53 for North Island herds, p=0.97 for South Island herds, and p=0.74 for all herds).

Table 2: Count (and row percentage) of North Island PCR positive herds in season 1 and season 2

	Season 2		
Season 1	Negative	Positive	Total
Negative	273	23 (7.8%, 5.0%-11.3%)	296
Positive	16	8 (33.3%, 15.6%-55.3%)	24
Total	289	31 (9.7%, 6.7%-13.5%)	320

Table 3: Count (and row percentage) of South Island PCR positive herds in season 1 and season 2

	Season 2		
Season 1	Negative	Positive	Total
Negative	42	2 (4.5%, 0.6%-15.5%)	44
Positive	3	18 (85.7%, 63.7%-97%)	21
Total	45	20 (30.8%, 19.9%-43.4%)	65

Incidence of new infection or clearance

There were 25 (7.4%, 95% CI 4.8% to 10.7%) new infections in initially PCR negative milking herds, and 19 (42%, 95% CI 27.7% to 57.8%) initially PCR positive herds that cleared infection from the milking herd. The proportion of herds clearing BVD virus infection was lower in South Island than North Island herds (14% vs. 67%, respectively, $p<0.001$), whereas new infection rates were similar in North and South Island herds (7.8% vs. 4.5%, respectively, $p=0.44$). This large difference in clearance rates resulted in the substantially higher prevalence in South Island herds (31.0% vs. 9.7%). Although the difference was not significant, the new infection rates mean that it would take 8.6 years until half of the remaining North Island herds had experienced infection, and 14.9 years until half of the remaining South Island herds had experienced infection.

If the virus introduction rate was similar in virus positive and virus negative herds, 7.8% of North Island herds and 4.5% of South Island herds that cleared the existing infection would have reintroduced a milking PI and appeared to maintain infection in a 12-month period. This is important for understanding the expected clearance rate if reintroduction is prevented through active control. The best estimate of the clearance rate where reintroduction is prevented in North Island herds is 67% / (100% - 7.8%) = 72%, and in South Island herds is 14% / (100% - 4.6%) = 15%. In the absence of reintroduction, it would, therefore, take approximately 4 years for 99% of North Island herds to naturally clear infection, and 29 years for 99% of the South Island herds to naturally clear infection.

Changes in BVD antibody category between seasons

The antibody categories of herds in the first and second season are shown in Table 4, and the change in antibody level between seasons is shown in Figure 1. The median change in antibody level across all herds was -0.08 (95% CI -0.10 to -0.06) units of S/P ratio. The median change for all herds not already in the "none" category in the first season and with a decreasing antibody S/P ratio between seasons (assumed not newly infected or re-exposed, 63% of herds) was -0.15 (95% CI -0.16 to -0.13) units. This trend for the antibody level to drop a little over half a category between seasons unless boosted by new exposure can be seen in Figure 1 and Table 4. At this rate of change, it would take approximately 7 years for a herd to go from the very high antibody category (e.g. 1.25 S/P ratio) to the no immunity category (<0.25 S/P ratio). Island did not have a significant effect on the change in antibody level between season (p=0.69). There were 52 (15.3%, 95% CI: 11.6% to 19.6%) initially PCR negative herds with an increase of greater than 0.1 S/P units of antibody between seasons, or no decrease and remained in the very high antibody category. The area under the receiver operating characteristic curve for predicting new infection (change to PCR positive) by the change in antibody level was 0.70. There was no cut-off value where both sensitivity and specificity were greater than 65%.

Table 4: Count of herds in each antibody category by season with row percentage in brackets

	Season 2					
Season 1	N	L	M	H	V	Total
None (N)	19 (59%)	10 (31%)	1 (3%)	0	2 (6%)	32
Low (L)	22 (27%)	51 (61%)	6 (7%)	3 (4%)	1 (1%)	83
Moderate (M)	4 (5%)	30 (39%)	31 (40%)	7 (9%)	5 (6%)	77
High (H)	0	5 (5%)	41 (42%)	36 (37%)	16 (16%)	98
Very high (V)	0	1 (1%)	3 (3%)	38 (40%)	53 (56%)	95
Total	45	97	82	84	77	385

Antibody levels: None (N) S/P ratio <0.25; Low (L) 0.25 - <0.50; Medium (M) 0.50 - <0.75; High (H) 0.75 - <1.0; Very high (V) ≥1.0

Relationship between bulk tank PCR and antibody level

There is a strong positive relationship between the BTM antibody level and a positive PCR status (Figure 2; Table 5). Most (85, 87%) of the PCR-positive herds also had "very high" antibody levels, though about half of all herds with "very high" antibody (90, 51%) were PCR negative. Only 11 (6%) of the "high" antibody herds were PCR positive, and 2 (1%) of the "moderate" antibody herds were PCR positive. A relatively small proportion of herds (79, 11%) had antibody levels below 0.25, and 41 (6%) had antibody levels below 0.2 indicating almost no exposure to BVD virus.

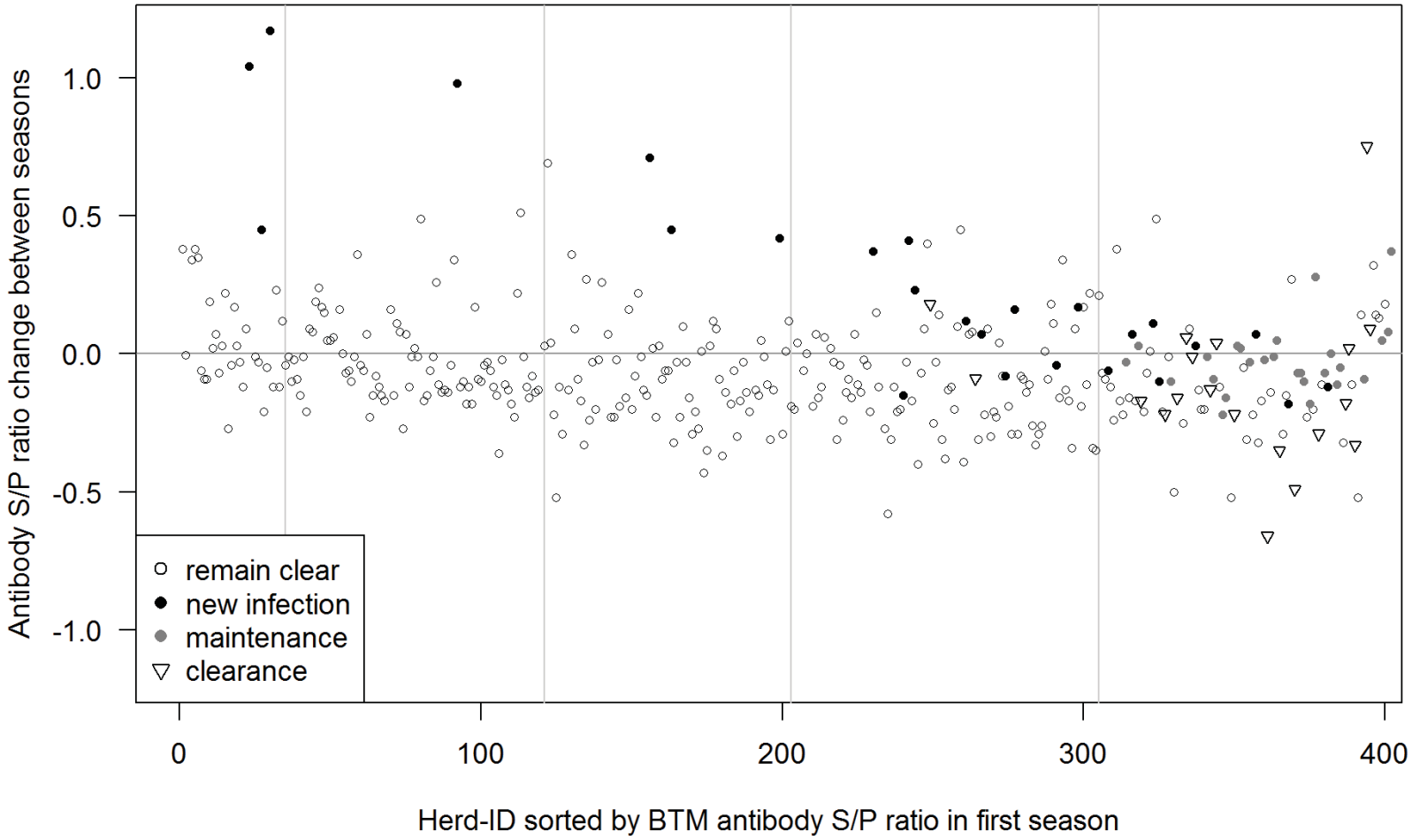

Figure 1: Bulk tank antibody level change between seasons with vertical lines corresponding with first season bulk tank antibody categories (none, low, moderate, high, and very high from left to right)

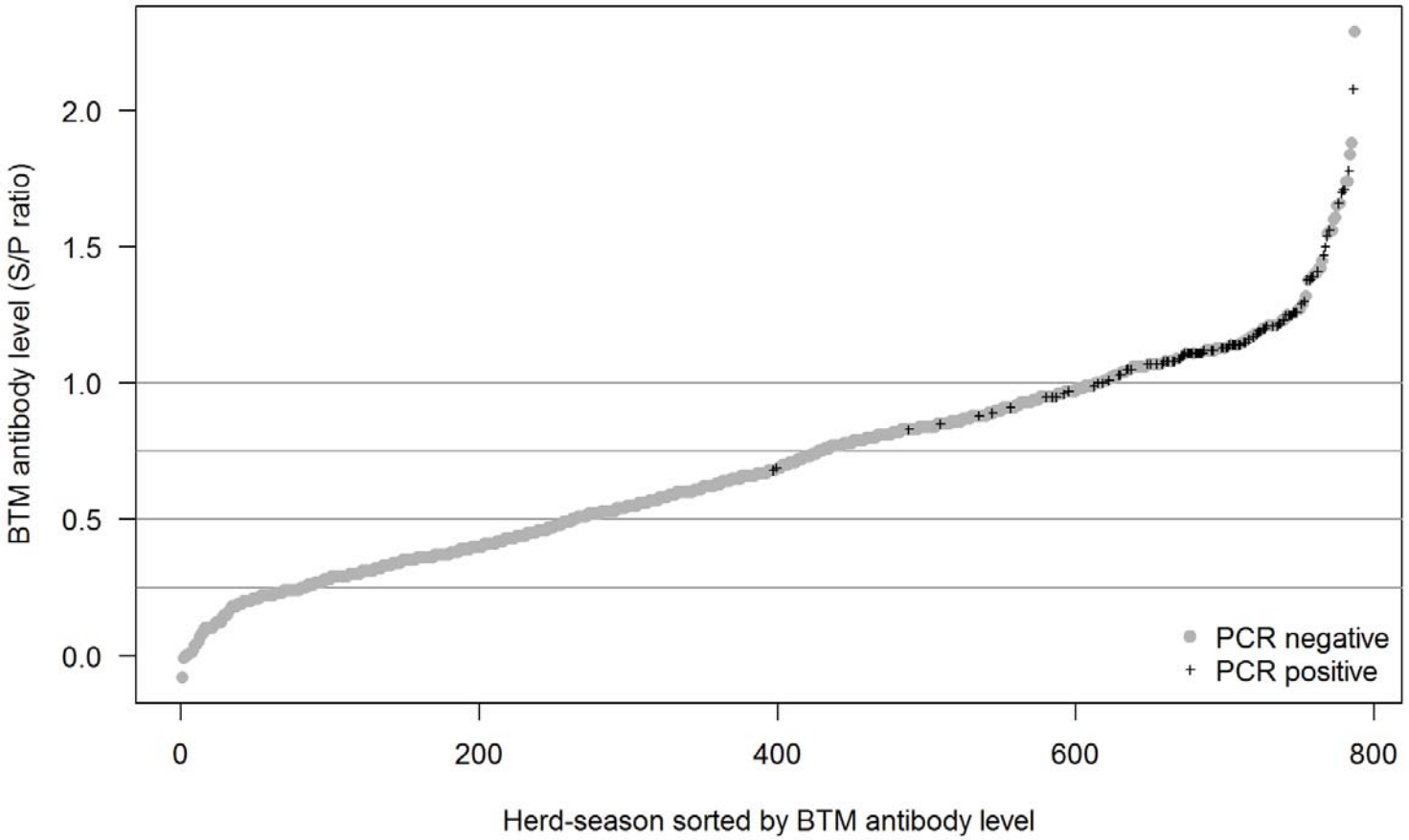

Figure 2: Antibody level and PCR status in all herd-season bulk tank samples (sorted by antibody level) with horizontal lines marking the cut-off values between "none", low, medium, high, and very high antibody categories.

Table 5: Count of PCR negative and positive herds by antibody category with row percentage in brackets

	PCR		
Antibody	Negative	Positive	Total
Very high	90 (51.4%)	85 (48.6%)	175
High	175 (94.1%)	11 (5.9%)	186
Moderate	163 (98.8%)	2 (1.2%)	165
Low	182 (100%)	0	182
None	79 (100%)	0	79
Total	689 (87.5%)	98 (12.5%)	787

High or very high BTM antibody, but PCR negative, herds were 3.4 times (RR, 95% CI 1.5 – 8.0) more likely to change to PCR positive status in the subsequent 12 months than herds with lower antibody levels. These high antibody herds had one extra apparent introduction per 10 herd seasons (risk difference = 0.1). Island did not have an effect on this relationship (Breslow-day p=0.55). The new infection rate by previous season antibody category was 9.4% (3/32) for previously "none" and PCR negative herds, 1.2% (1/83) for "low", 3.9% (3/77) for "moderate", 10.4% (10/96) for "high", and 15.4% (8/52) for previously "very high" antibody and PCR negative herds.

A comparison of status change between seasons based on antibody and PCR status is shown in Table 6.

Table 6: Comparison of status change between seasons with positive status defined as antibody S/P >= 1.0, or PCR positive.

	PCR				
Antibody	NN[1]	PN[2]	NP[3]	PP[4]	Total
NN[1]	263	1	6	0	270
PN[2]	25	9	3	4	41
NP[3]	10	1	11	0	22
PP[4]	16	8	5	22	51
total	314	19	25	26	384

[1] Negative in the first season and still negative in the second season
[2] Positive then negative indicating clearance of infection
[3] Negative then positive indicating a new introduction
[4] Positive then positive indicating maintenance of infection

Discussion

The high proportion of herds with evidence of past exposure (i.e. 94% of herds had BVD antibodies in the bulk milk) and the proportion of herds with high antibody concentrations (175 / 787 = 22% from Table 5) are in line with overseas studies (Paton *et al.* 1998; Stahl *et al.* 2002; Garoussi *et al.* 2008; Cowley *et al.* 2012; Humphry *et al.* 2012; Saa *et al.* 2012; Lanyon and Reichel 2014; Sayers *et al.* 2015). The estimates are also similar to previous New Zealand prevalence studies described in the introduction of this paper. The authors are unaware of any published studies reporting prevalence based on bulk tank PCR testing, or any studies reporting bulk tank milk status change between

seasons. This may be because bulk tank PCR testing is more difficult to interpret in year-round milking systems where there are non-milk cows on the property all year, and bulk tank antibody doesn't change as quickly or reliably as bulk tank PCR status with the arrival or departure of PIs. Although the incidence estimates from the current study may inform estimation of the patterns that might be seen in other systems, the rates of change will be highly management system dependent.

This data set was not generated by representative sampling across all districts of New Zealand so it is not appropriate to attempt to calculate a national prevalence. District prevalence information is available elsewhere (Voges 2008). Nevertheless, the district prevalences of this study were similar to those reported previously.

This study was observational, that is it did not alter any BVD control measures that were in place prior to enrolling or introduce new control measures. A detailed risk factor analysis will be presented in a subsequent chapter, but at the time of sampling for this study, the general awareness and understanding about BVD control was fairly low and interventions against BVD apart from bull testing were not common. Most herd owners engaged in BVD control measures other than bull testing had experienced a BVD outbreak in the past. The herd infection dynamics described in this study arise from a population with a low to moderate background level of BVD control.

Since antibodies are present in an individual for years after BVD virus infection (Fredriksen *et al.* 1999), bulk tank antibody levels indicate the history of exposure. Bulk tank antibody level can increase or be maintained at a high level due to current active infection but also due to exposure of stock while grazing away as heifers or dry cows, infection in the previous season (after the last test), indirect exposure, contact with neighbours stock, purchase of immune cows, or recent vaccination, all without infection being maintained on the farm. Bulk tank antibody levels decrease over time due to dilution with naïve replacements, and presumably due to decreasing antibody levels in individuals, so it is affected by the proportion of exposed individuals and the time since exposure as well as individual variation in the peak response and antibody change rate. There is also a lag of a few weeks for naïve cows (Niskanen and Lindberg 2003), or a few days for previously exposed cows before antibody level begins to increase so very recent exposures or introductions into mostly naïve herds, or introductions into already very high antibody herds may be missed if relying on antibody only.

The PCR virus test, on the other hand, detects current active infection in cows contributing to the bulk tank sample. A positive bulk tank PCR may rarely be triggered by transiently infected cows, but in the authors' experience and the advice from the laboratories performing the test, it usually indicates a milking PI (Ellison *et al.* 2011; Hill 2011). Non-milking PIs such as PI calves on the property will be missed by PCR (Mars and Van Maanen 2005) unless transiently infected cows cause a PCR positive. So the PCR status is an accurate measure of the current infection status of cows contributing to the bulk tank sample, and the bulk tank antibody test gives a good measure of historical exposure. Therefore, prevalence based on the bulk tank PCR test strictly refers to the prevalence of herds with active infection in the milking herd. The prevalence of herds with a PI animal of any stock class would also be of interest and relevant for control but could only be estimated from bulk tank testing using a combination of PCR and antibody status and antibody status change between seasons.

Misclassification of herd status is an important risk that could impact on all herd status and status change estimates. Because the bulk tank PCR usually only detects PIs that contribute to the bulk tank sample, a PI cow that is clinically ill and is excluded from the bulk tank milk could result in a negative result. Taking a second sample approximately two weeks after the first would in most instances have included cows that were not contributing to the first sample due to milk withholding

periods. However, a proportion of PI animals will be chronically ill and potentially excluded from both samples in which case the herd would have been misclassified as having no milking PI cows. The sampling strategy used in the current study focused on the window around the start of the seasonal breeding program. A negative herd BVD virus status could also have occurred where a PI animal was present in the herd but died or was removed before the first sample, or where a PI was introduced after the second sample. The rationale for focusing the sampling around the start of the seasonal breeding program was that this is the beginning of the high-risk period in terms of reproductive loss and creation of the next generation of PI calves. Although it is possible, experience with individual cow virus testing following a positive bulk tank PCR result indicate that it is rare for a transiently infected cow to cause a bulk tank PCR positive result (Ellison *et al.* 2011). In this case, the herd does have infection present at that time, but the infection would usually be short-lived unless the source of infection is a PI on the property that didn't contribute to the bulk tank sample such as a sick PI milking cow or a PI calf. Therefore, infection status as defined by the bulk tank PCR test is best described as the infection status of the milking herd around the start of mating.

The bulk tank antibody test does not depend on any one cow contributing to the sample, unlike the PCR test which depends on the PI contributing milk to the sample. However, only 49% of very high antibody herds were PCR positive so antibody does not appear to be a good indicator of a PI animal contributing to the bulk tank milk. This is not surprising since antibody levels persist in immune cows for years (Fredriksen *et al.* 1999). Even if half of the PCR negative very high antibody herds had a non-milking PI (Thobokwe *et al.* 2004), very high antibody level would still only be 75% specific for herds with any PI. Also, 13% of PCR positive herds had less than very high antibody level and some PCR negative herds with non-milking PIs would also have less than very high antibody levels. Therefore, although bulk tank antibody provides valuable information it is not as accurate or as easy to interpret for herd BVD virus infection status as the bulk milk PCR test in a seasonal calving system, particularly if the status of interest is the presence of a milking PI. Herds with a "very high" antibody level gain little from subsequent antibody tests until it decreases to a lower level. Bulk tank antibody tests for herds with lower antibody levels however can provide valuable information about exposure to virus (increasing antibody level between seasons) from sources that the PCR does not detect such as a PI calf where there is extensive exposure of the herd but no milking PI animals to cause a PCR positive bulk tank test.

There was a larger proportion of herds probably exposed (increasing antibody level) but not changing to PCR positive (15.3%, 95% CI: 11.6% to 19.6%), than herds that did change to become PCR positive (7.4%, 95% CI 4.8% to 10.7%). This may indicate that there were more external contacts (including heifers or cows while grazing away from the farm) and PI calves being born or bought than the rate of PI heifers or cows entering the milking herd. Since most heifers entering New Zealand milking herds were born to cows in the herd rather than purchased, it is likely that many of the PI heifers entering the milking herd were initially PI calves born on the property. Therefore, some of the apparent external contact rate may represent an earlier stage of the same process as some of the PCR status change rate.

Where PI calves or heifers die before entering the herd but cows are exposed to them after the start of mating, such as when cows travel along races past paddocks with PI calves, it is possible for herds to maintain infection in multiple generations of calves without having a PI in the milking herd. The loss rate for PI animals is estimated to be about 50% per year (Houe 1993), and some herds graze calves on the same paddocks as milking cows so contact is possible and this scenario may not be rare. Herds with this pattern of PI calf cycling would maintain high antibody levels for an extended period if there was enough contact between calves and cows, but not usually give a positive PCR result. Herds

wanting to control BVD need to use the bulk tank antibody test in addition to the bulk tank PCR test to indicate when this may be happening.

In this study, it was assumed that a herd changing from PCR negative to PCR positive between seasons represented a new introduction into the herd. However high or very high antibody herds were 3.4 times more likely to change to PCR positive than lower antibody herds. Although this may be because herds that recently introduced infection engage in practices that predispose them to BVD virus introduction, it could also be that they recently cleared infection from the milking herd but reintroduced infection via a PI replacement animal which was created during the previous period of infection. In the latter case, it could be considered maintenance of a previous infection rather than a new introduction if the larger view of all age groups in all locations is considered rather than just the milking herd.

The calculation of the expected clearance rate where reintroduction is prevented assumes all apparently cleared herds were truly cleared. The analytical sensitivity of the PCR test is very high (an unpublished in-house dilution test detected 1 PI in over 80,000 milk samples) so the main potential weakness from this assumption is a scenario where milking PIs exist in a herd but don't contribute to either bulk tank milk sample, and there are no transiently infected cows shedding virus at either sampling. Although this may be possible, especially considering that some PIs are more prone to severe or recurrent illness, it is likely to occur only rarely if at all in this data set so the calculated estimates should be a reasonable approximation. If it did occur, the clearance rate would be overestimated and the time to clear infection would be underestimated.

The clearance rate estimates also assume that the virus introduction rate is similar in currently virus positive and virus negative herds. It seems plausible however that currently virus positive herds may be at higher risk of virus introduction due to risky management strategies. This would be consistent with the higher rate of apparent introduction into high or very high antibody herds than into lower antibody herds although some of this may be due to the presence of non-milking PIs as previously discussed. If there was a higher rate of virus introduction into currently virus positive herds than estimated and this contributed to the apparent maintenance rate, the natural clearance rate from herds where reintroduction was prevented would be greater than estimated, hence the time to clear would be shorter. The degree of this effect cannot be assessed from this data because the probability of PI replacements re-infecting a recently cleared milking herd is not known. It is likely to be relatively common especially in large herds since PIs don't usually survive long in the milking herd and must be regularly replaced to maintain herd infection for the long periods of time seen. In the same way, herds that apparently cleared infection in this data set may have had replacement PIs coming through & therefore only temporarily cleared infection. If that is common and makes up a large proportion of the increased rate of apparent introduction into high antibody herds, then the time to clear infection when only preventing reintroduction will be greater than estimated.

The estimate of the clearance rates in herds where reinfection is prevented can contribute to herd owner decision-making. Since infection was likely to persist for many years in the large South Island herds, simply preventing a virus reintroduction will not be sufficient to eliminate the negative production effects of BVD virus in these herds. Effective vaccination or young calf screening for PIs would be expected to reduce the time it takes to clear infection since PIs have substantially greater loss rate than the rest of the herd (Houe 1993) so long-term maintenance of infection depends on regularly generating PI replacements.

There are a number of differences between the North Island districts and South Island districts in this study that explain differences in prevalence and rates of changing infection status. In New Zealand, most of the traditional dairy districts are in the North Island where the dairy farms tend to have relatively stable management, cow ownership, and herd size. Over the last two decades, many farms in the South Island districts in this study introduced irrigation systems and converted from farming sheep and beef cattle to large, intensive dairy herds. The median herd size in the South Island herds was 800 cows while the median North Island herd size was 265 cows. The mating period in South Island herds typically begins later than in most North Island herds due to different seasonal weather and grass growing patterns. This encourages the purchase of low-cost, late-calving cows from North Island farms. Many of these relatively large South Island dairy herds were formed by importing cows from multiple North Island farms. Some of the district differences in prevalence may be due to the importation of PIs, particularly where late-calving and cull cows were sourced from many different farms to form a herd. Herd size is likely to be important because maintenance of BVD virus infection in a herd depends on the survival or birth and retention of at least one PI and the greater the number of animals, the greater the chance of at least one PI being born and at least one surviving to enter the milking herd. There are other possible differences that may also be relevant including culling pressure, off-farm winter grazing of the herd and heifers, farm density and structure, labour levels and quality, and stock purchase rates.

The maintenance of infection appeared much more common in South Island herds resulting in a considerably higher prevalence. It is possible that more of the BVD virus exposures and introductions in South Island herds occurred in previously virus positive herds because of the higher prevalence. At a sampling interval of one year, this would have been misinterpreted as maintenance of infection instead of clearance between seasons followed by a new virus introduction before sampling in the second season. It seems plausible that there would be a correlation between current infection status and the probability of introduction of infection so the number of introductions could be even higher than observed. This would be important for modelling herd infection dynamics and relevant for control decisions, particularly for the probability of re-introducing BVD virus after having actively cleared the infection.

The high clearance rate in North Island herds has implications for control. It appears that most small herds will naturally clear infection within 3 or 4 years if they just prevent reintroduction without actively searching for the PIs and culling them. This reduces the potential benefit of actively clearing infection compared to large herds where infection was maintained for much longer periods since the small herds would soon clear infection even if not investing in active clearance while large herds would often remain virus positive for many years if they don't actively clear it. However, the cost of infection remaining in the herd for even a few years may be greater than the cost of clearing infection and a specific cost-benefit analysis is required to determine whether searching for PI's is likely to be cost effective for any herd size. Herd owner preferences and other non-economic issues will also be important in decision making. Such criteria include the perceived benefits of having a "clean herd" (predominantly personal satisfaction and reputation among peers), better animal welfare due to improved disease control, a sense of empowerment from taking active control of their BVD situation, or the social conscience factor since leaving infection in the herd is a threat to the stock of neighbours and other in-contact herds. This study also demonstrated that PCR negative herds have a considerable risk of becoming virus positive within a 12-month period, hence they cannot rely on their current status to indicate that they will remain virus negative. The new infection rate observed in this study is concerning and will support the potential value of investing in control measures. If herd owners – including immunologically naïve herds – want their herds to remain free of BVD, they will need to

reduce the risk of introductions by screening purchased stock as well as vaccinating the herd and/or screening replacements.

A comparison of status change between PCR defined status and antibody defined status demonstrated good agreement (79%) overall, but there was only 35% agreement after excluding herds where both tests agree there was no infection in either season. The largest disagreements occurred in herds that were not PCR positive in either season but did have high antibody in at least one season. These herds could have been exposed to BVD virus: in a previous season (especially antibody PN & PP herds); from a non-milking PI on the property; from neighbours' stock; during transport or while grazing away; or from a milking PI that didn't contribute to the bulk tank samples due to illness, very late calving, early dry off or death, or later purchase. This supports the view that the antibody test is valuable for indicating when exposure may have occurred without causing a bulk tank PCR positive result. An increase in bulk tank antibody level between seasons could indicate that calves should be tested since they are a possible source of infection for the milking herd, and if the herd was exposed in the previous season there may be PI calves born as a result. A bulk tank PCR positive result indicates that there may be a milking PI to be discovered and removed. So both tests have value and detect different types of infection (with some overlap), and potentially indicate different actions.

It is common for herd owners to assume that low or no antibody in the bulk tank indicates that they are at very low risk of exposure to BVD virus since there has apparently been no exposure for a number of years. That may be the case for some herds, but in the current study, approximately 10% of herds with no bulk tank antibody were BVD virus positive in the subsequent lactation. This rate of introduction may have been so high compared to "low" antibody herds due to chance since there were a small number of herds in this category, but it illustrates the fact that no antibody category was free from new infections. A lack of antibody in the bulk tank cannot be considered a strong predictor of low risk for new infection. Herd owners should, therefore, be made aware that a low bulk tank antibody level means a potentially severe impact if BVD virus is introduced and does not necessarily mean they are at low risk of such an introduction in the future.

The observed rate of change of the bulk tank antibody level in herds with a decreasing antibody level indicates that it would take an average of 7 years to go from the very high category to the "none" category. The replacement rate in New Zealand herds varies greatly but the median replacement rate in this study was 19.7% (95% range 5.7% - 30% in the current season). This replacement rate is consistent with the herd antibody change rate if individual cow antibody levels remained stable and most heifers entered the herd in a susceptible state, but neither of these conditions are likely to be accurate assumptions and it seems more likely that individual cow antibody levels decreased over time while at least some heifers came back from grazing immune. In the authors experience individual cow antibody level varies greatly between cows which is probably due to time since exposure among other factors, and many heifers entering herds will be immune due to exposure during transport or while grazing away. In this study, 81% of herds sent replacement calves away from the home farm for grazing until they returned to enter the herd as pregnant heifers. Most of these heifers would have grazed with stock from other farms including beef cattle in some cases. There is likely to be a high risk of contact with PIs for these young stock being transported in commercial stock trucks and comingling or grazing and moving around the farm near other stock. Only a subset of herds (herds where the antibody level was greater than "none", and the antibody level decreased between seasons) was included in the estimate of antibody change rate to avoid herds that experienced transitory infection and exposure to virus between seasons. Herds where there was no exposure but predominantly susceptible cows were culled between seasons, or where replacement heifers were immune may also have been excluded which would cause overestimation of the expected antibody

change rate in herds without exposure between seasons. Failure of the bulk tank antibody level to decrease over time could be taken as an indication that young stock were exposed before entry to the milking herd and may trigger a discussion or an investigation of grazing conditions. The authors could not find a comparable estimate of antibody change rate over time in the literature which may be partly due to the higher replacement rate in most other districts, variation in herd composition over time in year round systems, differences in test use, performance and categorisation elsewhere, and a preference for cross-sectional rather than longitudinal studies, among other factors.

It is concluded that BVD is widely present in the New Zealand dairy industry. However, there are substantial differences both in prevalence and in incidence of clearance of infection between districts. It is beyond the scope of the current study to define why these differences have occurred, but such differences have implications for the optimal testing and control strategy for BVD amongst districts. Although most PCR positive herds had “high” or “very high” antibody level, there was only moderate agreement between herd status as defined by the presence of BVD antibody in bulk milk and presence of BVD virus in bulk milk because they detect different types of infection. High antibody herds also had a higher risk of new introductions in subsequent seasons. Interpretation of the presence of virus is clear in that it indicates BVD virus infected animals contributing milk to the bulk tank. Given the apparently long half-life of BVD antibodies, the presence of antibodies indicates exposure of animals to the virus at some time point, and the change in antibody level between seasons can highlight exposure events that a PCR test cannot detect.

Chapter 3: Risk factor distribution and association with bulk tank Bovine Viral Diarrhoea (BVD) virus PCR and antibody status in New Zealand dairy herds

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Statement of contribution: Appendix 3

Introduction

Evidence of Bovine Viral Diarrhoea (BVD) virus was first detected in New Zealand in 1966 (Fastier and Hansen 1966), but there have been no studies on BVD virus transmission in the New Zealand farming system. New Zealand dairy farms differ from most other dairy production systems worldwide and this contributes to uncertainties about infection dynamics and the associated risk factors. Over 95% of New Zealand dairy farms have a strictly seasonal calving pattern (Verkerk 2003; Blackwell *et al.* 2010) with typical mating periods of 10 - 17 weeks. Most cows that fail to conceive during this period are culled at the end of lactation. Most cows in a herd are dried off on a specific calendar date or over a few days for larger herds. Dry periods last 6 to 12 weeks during which cows may be sent away from the home farm for grazing. Cows spend all year on pasture and pasture or products grown on the home farm like grass silage make up 79% of total feed eaten in New Zealand (D. Silva-Villacorta[4] personal communication, 7th July 2015). Commercial herd sizes vary from 100 to over 1,500 cows. Most cows walk to the milking shed and back to pasture twice a day, usually 1-4 km a day. Replacement calves are typically grazed away from the milking farm until they return pregnant and close to their first calving, but they remain on the farm through the mating period in some herds. A "sharemilking" system exists in New Zealand where the income from the milk is shared between the farm owner and the person managing the farm (known as a "sharemilker"). The proportion of the income a sharemilker receives varies depending primarily on what proportion of the cows they own (from none to all of the cows) and can change over time as the proportion of cows owned by the sharemilker increases. Cows are the main reserve of capital for a sharemilker and there is an incentive to increase herd size with an excess of replacement calves and low culling rate. This system is associated with increasing herd size, reduced culling rate, higher replacement and purchase rates, and regular herd movements as herd size exceeds the capacity of the current farm or as contracts expire. In pasture-based, seasonal grazing systems, cows tend to produce less milk per day for shorter lactations than in more intensive year-round systems but can be fairly long-lived under New Zealand conditions where cows sometimes reach productive lifetimes of greater than ten years.

BVD infection is usually spread and maintained by contact with persistently infected (PI) animals (Houe 1999). Stock purchase is a commonly identified risk factor (Houe *et al.* 1995a; Valle *et al.* 1999; Presi *et al.* 2011; Gates *et al.* 2013; Graham *et al.* 2013; Williams and Winden 2014) which will include the risk from purchasing PIs and cows carrying a PI fetus ("Trojan cows"). Virus transmission from neighbours across the fence has been identified as an important risk (Valle *et al.* 1999; Ersboll *et al.* 2010) and had a similar explanatory power to stock movement in one study where the risk of virus

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introduction was examined (Gates *et al.* 2013). Contact of cows over the fence are of particular importance in New Zealand where cows are on pasture all year, hence fence lines are longer and time spent grazing along a boundary is greater than in partial indoor systems, and a large proportion of dairy farms share a boundary with other dairy farms. Herd size has been strongly associated with infection risk (Houe *et al.* 1995a; Presi *et al.* 2011; Humphry *et al.* 2012; Graham *et al.* 2013). Agistment (moving stock away from the home farm for grazing) has also been associated with exposure to BVD virus (Valle *et al.* 1999; Presi *et al.* 2011; Gates *et al.* 2013). The main risks relevant to the New Zealand system were therefore hypothesised to be herd size, stock purchase, contact with neighbours' stock, and movement of stock off the farm and back again. Other potential risks include aerial spread and indirect spread via fomites, shared facilities, or trucking. However, the potential risk from such factors are likely difficult to measure, are usually considered to be less important, and no estimates of this risk have been published.

Understanding the relative importance of risk factors for the introduction of BVD virus or maintenance of infection is fundamental to planning control at a herd and a national level. This study was therefore designed to quantify the potential relationship between management factors and BVD virus infection status defined by bulk tank antibody and bulk tank PCR test results.

Materials and methods

We conducted a cross-sectional and prospective cohort study in a subset of herds, with herd as the unit of interest.

Herds

A convenience sample of clients (n = 206) of a Taranaki vet practice (Eltham district veterinary services, Eltham, New Zealand; approximately -39.36 to -39.48 latitude, and 174.08 to 174.40 longitude), who completed an initial questionnaire and reported that they were planning to stay on the farm for at least the next two lactation seasons, were enrolled for bulk tank BVD PCR and antibody testing in 2007/2008 lactation. Of these herds, 193 (94%) repeated the questionnaire and were re-sampled in the 2008/09 lactation. This process of conducting questionnaires and bulk tank testing was repeated in 2009 / 2010 and 2010/2011 with 63 clients of a Waikato vet practice (all re-tested in 2010), and with another 133 herds (33 / 32 from Eltham, 35 / 33 from Hamilton, 33 / 33 from Ashburton, and 32 / 32 from Oamaru, in the 2009 / 2010 seasons respectively). Farmers of the latter (133 herds) group were recruited from the National Herd Fertility Study (Brownlie *et al.* 2011). There were 25 herds from Eltham enrolled in all 4 seasons while most other herds contributed data to two seasons. Further detail of the herds is provided in chapter 2.

Testing

Bulk tank milk (BTM) sampling was conducted twice in each herd approximately two weeks (median 14d, 95 percentile range 12d – 28d) apart. The first sample was collected when herd managers estimated in advance that all of the first calving cows and "most" of the rest of the herd would have calved. While some first calving cows may not have calved by the time of the second sample, this would likely be rare and have little impact on the inferences. Milk samples were the duplicate representative bulk tank samples collected by milk truck drivers at each milk collection for component and microbiological analysis. The samples were chilled and were tested within 3 days of collection in most cases and less than 5 days for all samples. Samples were tested using the MagMAX™-96 Viral RNA Isolation Kit for RNA extraction, and the PCR kit VetMAX™ - Gold BVDV PI Detection Kit for the presence of RNA. All BTM samples were also tested for antibody using the IDEXX BVDV Total Ab ELISA Test using an overnight incubation. All testing was conducted by a commercial laboratory (Genemark Animal Health, Hamilton, New Zealand).

Any signal up to 40 cycles was considered positive for the real-time PCR and if either sample was PCR positive, the herd was classified as virus positive for the season. The ELISA S/P ratio (sample to positive ratio) was interpreted as being equivalent to the concentration of antibody in BTM. The larger of the ELISA results from the two sampling times within season was used as the herd antibody level for the season.

Questionnaires

Questionnaires were sent to farmers by mail and a combination of prize draws and 2-weekly phone reminders were used to encourage completion and return for Eltham in 2007 and 2008, and for Morrinsville herds. Farmers of the National Herd Fertility Study were interviewed by trained technicians as part of a larger questionnaire. The questions were the same except for some of the herd details that were drawn from existing data generated for the other study. Calculations from multiple inputs and conditional logic checks were built into the database to identify apparently erroneous answers or data entry errors. Any unusual, implausible, or inconsistent responses, answers that contradicted the vet's expectation for a farm, or missing values were followed up with phone calls by the investigators where possible and confirmed or corrected. This resulted in response proportions of 88% in Eltham, 54% in the Morrinsville district, and 99% for farms of the National Herd Fertility Study. Among farms included in the analysis, there were less than 2% missing or unlikely data values in the questionnaire data.

The questionnaire (Appendix I) sought data including herd details such as herd size, proportion of herd replaced with young stock on an annual basis (replacement rate), management group (mob) sizes, area upon which cows and replacement animals were grazed (land area), breed, labour, ownership structure, key seasonal dates (for example planned start of the seasonal calving and breeding programs), pregnancy and abortion rates, BVD history and vaccination practice; stock movements by class; if and when calves leave the home farm; replacement calf sources (i.e. if calves are kept from potentially higher risk classes); calf grazing system at home including hospital paddock policy; breeding bull purchase (number, age, duration of stay, testing, management on farm); cow purchase and testing; carry-over cow policy (cows that were non-pregnant at the end of the seasonal mating period and retained for an extra season); quarantine policy; degree of subdivision; and boundary risk (proportion of paddocks where over the fence contact with neighbours stock was possible, number of neighbours with stock, and extent of contact when possible).

Analysis

Data were entered and stored in a relational database (Microsoft Access), validated using routine procedures (missing values, min/max, and outlier analyses), and analysed using R 3.1.2 (R Core Team 2014). Risk factors associated with PCR status, bulk tank antibody level, and incidence or clearance of infection were the primary outcomes of interest in this study.

Descriptive analysis included the distribution (min, max, mean, median, 95% range, or category percentages) of continuous variables or counts by level for categorical variables of interest.

Multivariable regression models.

Bulk tank PCR status (positive/negative) was analysed by logistic generalised estimating equation (GEE) regression with herd as a subject effect to account for repeated BTM tests of the same herd. BTM antibody level was treated as a continuous outcome and analysed by linear GEE regression, again with herd as subject effect. Logistic regression models for changing PCR status between season were evaluated where the subset of herds initially virus positive (for clearance model), or initially virus negative (for new infection model) were included in each model. Due to complete separation for

vaccination and new infection status, a Monte Carlo Chi-square test was used to assess this relationship. Status change regression models based on antibody level were not attempted because antibody level can increase due to many factors other than the introduction of a PI such as vaccination, herd composition changes, milk volume, and colostrum contamination, and antibody level may not decrease substantially between seasons despite clearance of infection.

All variables related to the outcome with a single variable regression $p \leq 0.20$ were considered for inclusion in multivariable models. The best fitting or most biologically meaningful of highly correlated predictor variables – such as various measures of herd size – were included in an initial full model. Variables were then dropped in a reverse step-wise manner until all remaining variables were significant at $p \leq 0.05$. Variables dropped in the process were then re-entered one at a time to evaluate confounding. Previously excluded variables including highly correlated alternatives were then tested one at a time for significance in the intermediate model. Finally, all alternative variables not previously tested were substituted for their alternative and the best fitting was retained with consideration for simplicity of interpretation. Variables tested in an intermediate model were retained if significant at $p \leq 0.05$, or if evidence of substantial confounding was observed (change in other estimate value of 30% or more). Continuous variable linearity was tested using polynomials and higher order polynomials were retained if they improved the fit to the data. Biologically plausible interactions among significant variables were tested and retained if they added substantial information (QIC). Linear regression models were examined for normality and homoscedasticity using residual plots and any marked departures reported.

Results

Descriptive results

In this study 481 (61%) herds managed adult dry cows (winter grazing) on the land used to provide pasture for the milking cows, 170 (22%) grazed dry cows on land remote from the milking area where over the fence contact with other stock was possible, 87 (11%) grazed dry cows in isolation from other stock, and 49 (6%) were mixed with stock from other farms (1 herd with missing data). There were 430 (55%) herds that rotationally grazed calves on the milking herds paddocks (staggered from milking herd) while they were at home, 258 (33%) grazed them separate from the milking herd paddocks but still on the same farm, 72 (9%) managed the calves by grazing in multiple small groups on the pasture used by the milking cows (a few calves would be mixed with the milking herd between each milking but remain in the paddock after the milking herd leaves), and 23 (3%) used some other system (5 herds with missing data).

Most herds (612, 79%) purchased or leased bulls for one seasonal breeding period, 133 (17%) herds kept bulls for 2 to 3 breeding periods, and 25 (3%) herds kept bulls for 4 or more breeding periods (18 herds didn't answer). Farmers of 472 (63%) herds reported that breeding bulls were tested for BVD virus before arriving on farm, 9 (1%) tested them after arrival, 189 (25%) reported they were never tested, 60 (8%) did not know, 23 (3%) did not use any breeding bulls, and 35 (5%) did not answer the question.

There were 496 (63%) farmers who did not purchase any cows in the current or previous season, 89 (11%) who purchased cows up to 5% of herd size, and 201 (26%) who purchased many (i.e. equivalent to >5% of herd size) cows (2 herds missing). The sample included 31 (4%) herds that were defined as newly established as more than half of the cows were purchased in the current season. Fifty (6%) farmers reported they tested at least some of the cows for BVD virus at the time of purchase if they purchased stock (included herds that didn't purchase cows in the current or previous season but had established that policy), 292 (37%) never tested purchased cows, and 446 (57%) had a policy of

not buying any cows (none missing). There were a minority of farmers (n=157; 20% of all herds, 54% of those purchasing cows) that reported that they planned to keep calves from purchased cows (none missing).

While on average, 38% of farmers (n=297) kept replacement calves from heifers, this varied greatly by district (Morrinsville 26%, Eltham 31%, Hamilton 43%, Ashburton 69%, Oamaru 78%). There were no questions about cows that were retained in the farm system despite having failed to get pregnant in one seasonal breeding program ("carryover" cows) in the 2007/2008 questionnaires. After including this question in the 2009/2010 questionnaire, 186 (48%) farmers reported that they currently kept carryover cows, and 98 (25%) farmers sometimes kept calves from carryover cows when they kept carryover cows (46 herds that reported sometimes keeping calves from carryover cows did not currently have any carryover cows). The median, mean, and maximum number of carry-over cows among herds with any was 10, 17, and 196 cows respectively, and the median, mean, and maximum number of carry-over cow as a percent of herd size was 2.3%, 3.3%, and 18.4% respectively.

While about half of all farmers (n=415; 53%) were unable to indicate how many cows made contact over the fence with neighbours' stock when such contact was possible, 109 (14%) farmers reported that cows had no interest and did not make contact when they had the opportunity, 140 (18%) reported that a small number (1-5) of cows made contact, 59 (7%) reported 6 to 20 made contact, and 64 (8%) reported more than 20 made contact. Many of the latter category said, "*most cows crowded around and had a turn*".

At the time of this study, calves in 319 (40%) herds were vaccinated against BVD virus, 103 (13%) farmers vaccinated animals between one and two years of age ("heifers"), 63 (8%) vaccinated the milking herd, 341 (43%) vaccinated any female breeding or replacement stock, and 62 (16%) reported that all of their breeding bulls were vaccinated (none missing, question about bull vaccination not included in 2007/2008).

Herd characteristics and performance parameters are summarised in Table 7.

Table 7: Summary of selected continuous variables

Name	n[1]	min	5%[2]	25%[2]	50%[2]	75%[2]	95%[2]	max	mean
Herd size	786	62	135	212	295	448	944	1,575	379
Replacement rate (%)	783	0	10.5	17.2	19.7	22.2	26.9	40	19.4
Stocking rate (cow/ha)	786	0.9	1.2	2.8	3.1	3.4	4.0	5.6	3.1
Total mating length (d)	783	46	71	84	92	104	122	195	95
Cows per labour unit[3]	787	39	79	120	145	174	219	300	147
Weeks calves at home[4]	782	0	1	6	14	29	33	37	17
Cows per bull[5]	756	18	49	79	102	131	193	350	110
Proportion boundary[6]	787	0	0	0.08	0.17	0.36	0.75	1	0.25
Number of neighbours[7]	788	0	0	1	2	3	5	16	2.4

[1] Number of responses out of 788 herd-seasons (the difference from 788 is the number of missing values, or not applicable for cows per bull).

[2] Quantiles – percentage of herds with the given value or less

[3] The number of labour units was the answer to the question "how many full-time equivalent labour units are there working on your farm (including yourself, partial units OK)?"

[4] How long calves were managed on the milking area (includes time in rearing sheds). For the 19% of herds where calves remained on the home farm throughout, this is how long until they were shifted to dedicated calf/heifer areas of the farm.

[5] A measure of bull power for the herds that used bulls for part of the mating period (32, 4% didn't). Note that many cows will be pregnant when the bulls are introduced after a median 40 days of artificial breeding (5% - 95%: 25d – 60d).

[6] The proportion of paddocks where nose to nose contact over the boundary fence with neighbours stock is possible.

[7] Number of neighbours with cattle with a shared boundary where nose to nose cow contact over the boundary fence is possible

Bulk tank PCR status

The prevalence by district and season is presented in Weir et al (chapter 2).

The odds of being bulk tank PCR positive increased 1.34 times with every 100 cow increase in herd size (Table 8). The odds of a positive bulk tank PCR increased by 2.18 times (118% increase in the odds) if the number of untested purchased cows in the current and previous seasons combined was equivalent to the number of cows in the herd (the proportion of the current herd size purchased over the last 2 seasons), compared to herds that purchased no cows or tested all purchased cows. There were no other explanatory variables for BVD virus presence in the bulk tank in the final model.

Table 8: Generalised estimating equation logistic regression model of bulk tank BVD PCR status

	OR (95% CI)	Estimate	Std.Error	p
Intercept		-3.313	0.261	<0.001
Herd size last year (/100 cows)	1.34 (1.22-1.48)	0.294	0.050	<0.001
Bought-in[1]	2.18 (1.10-4.34)	0.779	0.351	0.027

n=781; Scale parameter = 0.976 (std. error: 0.276); correlation parameter (AR1) = 0.291 (std. error 0.111, 373 clusters). Predictor variables are continuous and approximately linear on the logit scale.

[1] Bought-in is the cumulative proportion of the herd bought but not tested "last year" and "this year". Where bought cows were tested for BVD virus, the value is set to zero.

Interpretation: After accounting for herd size, the odds of a positive bulk tank PCR increased by 2.18 times if the number of untested purchased cows in the current season and previous season combined was the same as the number of cows in the herd last season, compared to herds with no purchases in the last 2 seasons, and this was significant (p=0.027). To estimate a different proportion such as 10% untested purchased cows calculate exp(0.779 x 0.10) = 1.081 times the odds, or 8.1% higher odds of being PCR positive.

Bulk tank antibody status

Herds that vaccinated adult cows had a bulk tank S/P ratio 0.225 units higher on average than those not vaccinating (Table 9). All other districts had higher S/P ratios than Eltham, with Oamaru having the highest S/P ratios. Keeping replacement stock from purchased pregnant cows increased the bulk tank antibody level by 0.077 units on average. The bulk tank antibody level increased by an average of 0.112 units if the number of untested purchased cows in the current and previous seasons combined was equivalent to the number of cows in the herd, compared to herds that didn't buy any cows in the last 2 seasons or tested all purchased cows. The S/P ratio increased by 0.025 units on average for every 100 cows in the herd. Farmers that reported that many cows (>20 cows) made contact with neighbours' stock over the fence when they had the opportunity, had a bulk tank antibody level 0.129 units higher on average. Owner-operator herds had the lowest average antibody level, followed by higher order share milkers at 0.058 units higher, and lower order share milkers had the highest average bulk tank antibody level at 0.085 units higher than owner-operators. Finally, herds that grazed cows away from the home farm with stock from other farms in the winter had the lowest average bulk tank antibody level at 0.022 units lower than herds that grazed dry cows on the same farm (home) as they were milked, and herds that grazed dry cows away but not mixed had the highest average antibody level at 0.049 units higher than herds that kept their dry cows at home in the winter.

Table 9: Generalised estimating equation linear regression model of bulk tank BVD ELISA sample to positive (S/P) ratio

	N[1]	Estimate (95%CI)	Std. Error	p
Intercept	781	0.453	0.034	<0.001
Cows vaccinated	781	0.225 (0.098-0.351)	0.065	<0.001
District				
Eltham	465	Ref	Ref	<0.001
Morrinsville	124	0.147 (0.070-0.225)	0.039	<0.001
Gordonton	68	0.065 (-0.035-0.165)	0.051	0.202
Ashburton	60	0.119 (-0.032-0.270)	0.077	0.122
Oamaru	64	0.329 (0.183-0.475)	0.075	<0.001
Calves kept from bought cows	781	0.077 (0.037-0.118)	0.021	<0.001
Bought-in[2,3]	781	0.112 (0.041-0.184)	0.036	0.002
Herd size last year (/100 cows)[2]	781	0.025 (0.008-0.042)	0.009	0.003
Contacts over the fence[4]	781	0.129 (0.015-0.243)	0.058	0.027
Cow ownership				
owner	488	Ref	Ref	0.027
Lower order sharemilker	132	0.085 (0.011-0.159)	0.038	0.024
Higher order sharemilker	161	0.058 (-0.005-0.122)	0.032	0.071
Cow grazing				
home	480	Ref	Ref	0.044
away	252	0.049 (0.001-0.097)	0.024	0.045
mixed	49	-0.022 (-0.095-0.051)	0.037	0.556

[1] number of herd seasons
[2] Continuous
[3] Bought-in is the cumulative proportion of the herd bought but not tested "last year" and "this year". Where bought cows were tested for BVD virus, the value is set to zero.
[4] When contact over the fence with neighbours stock is possible, many cows (more than 20) actually do make contact (62 herd seasons, or 8%, reported this result). This is compared to herds that reported fewer than 20 cows made contact when it was possible (198, 25%), no cows made contact despite it being possible (109, 14%), or they were unable to comment (412, 53%)

Interpretation: After accounting for district, calves kept from bought cows, bought risk, herd size, contacts, cow ownership, and cow grazing, herds that vaccinated cows had an antibody S/P ratio 0.225 units higher on average of and this effect was highly significant (p<0.001).

Six herd seasons were excluded due to missing data.

Status change between seasons.

Increasing herd size (/100 cows) was associated with 0.64 (95% CI: 0.48-0.85) times the odds of clearing infection (p=0.002, SE = 0.145, intercept 2.14), and was the only variable significantly associated with clearing infection. New infection of previously virus negative herds did not appear to be associated with cow vaccination (Monte Carlo Chi-square p=0.25) even though none of the 25 vaccinated herds were newly infected while 8% of the unvaccinated ones were. This complete separation for vaccinating herds prevented modelling by frequentist methods, and a larger sample size for newly infected herds may have revealed a significant effect. No variables were significantly associated with new infection risk in this data set.

Discussion

This prospective observational study has demonstrated that presence of BVD virus is associated with increasing herd size and purchase of untested stock. The level of BVD antibody in bulk milk was higher in larger herds, in herds using a BVD vaccine, where calves were kept from purchased cows, where there was cross-fence contact with other stock, where cows were owned by one party and the land by another, where cows were grazed away from the home farm and varied with district within New Zealand. Increasing herd size was associated with a lower probability of BVD virus disappearing from the herd.

The herds in this study were a convenience sample. Some herds self-excluded by not returning questionnaires or invitations or were not selected for the National herd fertility study because their veterinarian thought they were less likely to comply with study requirements. These herds may have been systematically different to the herds included in this study. For example, these herd managers may have had a weaker relationship with their vet and less interest in disease control so may have been more likely to experience BVD virus incursions. They may also have had a lower level of understanding of the risks of BVD. One criterion for inclusion in this study was the managers planned to remain at the same location through to the end of the next season. Most stock managers that were planning to move at the end of the first season may have been sharemilkers who may also be more likely to increase herd size. This condition may, therefore, have introduced some bias in some measures such as replacement rate and stock purchase rate. Although the prevalence of outcomes and the distribution of some risk factors in the study herds may differ from the prevalence and the distributions in the population, the relationships between risk factors and the outcomes of interest are not likely to differ so inferences about the association between BVD virus infection and risk factors may be applied to other New Zealand herds.

Bulk tank PCR status (detection of the virus in bulk tank milk by PCR) is the most specific and easily interpreted outcome measure but does not detect past infections or infection in non-milking stock (chapter 2). Some of the risk factors were likely consistent across time such as district or potential for cross-fence contacts, and may only be associated with a milking PI if an exposure event at least 3 years earlier led to the birth of a replacement PI. Therefore, there may not be close temporal association of exposure events with the current state of these risk factors. Risk factors associated with an increased risk of exposure would only be significantly associated with current PCR status if they had a high probability of causing infections across multiple years, or if herds were able to maintain the infection for long enough after an introduction that a substantial proportion of herds with that risk factor were still PCR positive at the time of testing. The presence of a PI in a specific herd varied across time (chapter 2), with many herds changing status from PCR positive to PCR negative between lactations. In contrast, even if herds did not produce a replacement PI after exposure or cleared infection quickly, bulk tank antibody level would still be elevated for a number of seasons so an association would be considerably easier to demonstrate. There were only 98 (13%) PCR positive herd seasons and it is a binary outcome so the power of this regression model was also limited compared with the continuous antibody level outcome. Many of the variables were recalled by the herd manager, rather than measured directly, so there is a risk of recall bias or a lack of specificity of the definitions which could reduce the apparent association of these factors with BVD status. These limitations might explain why only two factors significantly contributed to explain the seasonal BTM-PCR status (Table 8). However, there were many significant variables in the antibody status model (Table 9), and since misclassification tends to reduce the significance of association, those variables are unlikely to be substantially misclassified in this way.

Bulk tank antibody level is a measure of past exposure and cows can remain antibody positive for at least three years after an exposure (Fredriksen *et al.* 1999). There are numerous methods for the bulk tank antibody level to increase or be maintained at a high level other than a PI in the milking herd (chapter 2). While the antibody concentration in BTM correlates highly with the antibody prevalence of cows (Beaudeau *et al.* 2001b), it may also be affected by time since individuals were exposed. Little is known about the individual antibody decay rate. Due to the wide range of replacement rates, and the risk of young stock being exposed while away grazing, the expected dilution effect on bulk tank antibody level from naïve young stock replacing previously exposed cows may be quite variable. In the authors' experience, BTM antibody may remain high for a number of years in some herds with no evidence of active infection, while in other herds it may decrease substantially between seasons once infection has been cleared. Thus, the regression model effectively modelled the effect of predictors on the combined effect of antibody decay rate, time since exposure, and proportion of cows exposed to the virus.

Herd size had a significant influence in all models indicating that large herds were more likely to be infected (as defined by PCR status and antibody status), and less likely to clear infection (from the clearance model). Herd size had changed from the previous season for 57% of herds and 19% changed by more than 25 cows, so many of these herd sizes were dynamic. Recall bias should not have a large effect on this variable because of the importance of total cow numbers for farm management. The herd size last year was the best fit, even with the bought cow variable removed, which may indicate that herd size was related to the maintenance of infection more than new virus introduction. If herd size was related to new infection risk (an event in the current season), the current herd size should be the best fit, but if it is related to maintenance of infection (a status carried over from the previous season), the herd size from the previous season could be the best fit as seen in this study. In addition, herd size was associated with the chance of virus positive herds clearing infection (larger herds much less likely to clear, $p<0.001$ in the BVD clearance model), and was not associated with the chance of initially virus negative herds becoming PCR positive ($p=0.62$ in the new infection model, not shown). The most important issue related to herd size may be the number of calves born on farm and retained in the herd for replacement because this would increase the chance of at least one PI being kept and surviving to maintain infection. The number of naïve cows available to generate more PI's may also increase the chance of maintenance of infection. Herd size is not useful as a tool to reduce the risk of BVD, but this result can inform decision-makers and modellers.

The total proportion of bought-in cows in the herd over two subsequent seasons that were not tested was a significant risk for both high BTM antibody, and a positive BTM-PCR. Buying in cows is known to be one of the most important risk factors for BVD virus introduction (Houe *et al.* 1995a; Valle *et al.* 1999; Presi *et al.* 2011; Gates *et al.* 2013; Graham *et al.* 2013; Williams and Winden 2014). The stock purchase risk may be associated with the purchase of immune cows exposed at another site for the bulk tank antibody level model, as well as the risk of a new exposure. The variable with bought proportion set to zero if cows were tested for virus was a better fit than the straight proportion bought which supports the concept that testing bought cows can be an effective control measure. This risk factor would have contributed to modelling some of the effect from PI fetuses since the risk of bringing in a PI fetus will be strongly associated with the number of bought cows. Testing bought cows cannot protect against PI fetuses carried by non-PI cows, however, since they will be virus negative when tested. The improved fit where purchased stock were not considered a risk if tested may indicate that purchasing PI cows represent a greater risk than purchasing a non-PI cow carrying a PI fetus. If the purchase of immune cows carrying a PI fetus was a greater risk, the straight proportion purchased should have been the best fit.

The policy of keeping calves from bought-in cows was associated with bulk tank antibody level. This may be due to the introduction of cows carrying PI calves, survival of whom would result in the exposure of replacement and potentially lactating stock to virus. This variable is also likely associated with the stock purchase variable due to the risk of purchasing immune cows applying to both variables. This association may have led to an underestimation of the effect of keeping calves from bought stock. Given the high PI loss rate (Houe 1992), the proportion of adult PIs must be much lower than the proportion of cows carrying PI fetuses, hence if PI cows are a greater risk than cows carrying PI fetuses, this indicates that the loss rate for PI fetuses and calves is greater than their increased number. That is reasonable since on average, approximately 80% of calves are not retained on-farm more than 4 days due to being male or later calving female so there is a high chance of incidentally culling PI calves before they can cause many infections.

Eltham had the lowest antibody level even after accounting for the other risk factors in the models. The order of the other districts varied depending on which outcomes were modelled except that Oamaru was consistently the highest risk district. District was significantly associated with BVD status after accounting for other known risk factors including herd size which indicates that there was something else about district that affected BVD status. The median herd size for Eltham, Morrinsville, Hamilton, Oamaru, and Ashburton was 254, 260, 385, 698, and 805 cows respectively so there is a general trend of a district effect with increasing average herd size, but with substantial variation in district coefficients from this general trend. The South Island districts (Ashburton and Oamaru) have a number of management and typical herd history differences (how the herds were formed, and how recently this occurred) from the North Island districts described in chapter 2 which may help to explain a higher prevalence of infection as large new herds are formed. Once a higher prevalence has been established it would modify the risks from neighbours, transport, and agistment which may account for some of the district effect and positively feedback to maintain or increase it further to a higher prevalence. To test whether the local prevalence explained the effect of district, the district variable in the final model was replaced with the district prevalence for that season as a continuous value (where all herds from each district have the same value in a given season). This alternative district variable was associated with bulk tank antibody level at p=0.02 (not shown) which indicates that this may be an important part of the district effect but does not account for all of it. It is also possible that there was an unrecorded variable related to BVD status which varied by district, and it is possible that the district variable captured some of the effect of indirect spread such as aerial or fomite-mediated spread.

Vaccinating the milking herd was strongly associated with the bulk tank antibody level. Vaccination directly increases the antibody level if administered before milk sampling which would often be the case because vaccination must be done before the start of mating to ensure protection through the period when PI fetuses can be created and this coincides with the time of bulk tank sampling. There were 59 (7.6%) herds that vaccinated the milking herd and in the authors' experience, the decision to vaccinate the herd was often made in response to an outbreak. Vaccination was therefore both a cause of higher antibody levels (not infection) and a response to recent BVD in some herds. Vaccination would not contribute to PI loss rate, but it should reduce the probability of a replacement PI being created before a PI is culled or dies. However, vaccination did not appear to speed clearance of infection in this dataset (p=0.31 when forced into the clearance model, not shown). This may be partly due to a large number of relatively small herds (where PI replacements were unlikely to be created anyway) masking a potential effect in larger herds with a significant risk of maintaining infection. It may also be due to the relatively low power of this model.

Having fewer labour units for a given number of cows appeared to increase the risk of exposure to BVD virus even after accounting for herd size and district. This may be an indicator of the style of management such as the number of mobs herds are managed in, the level of care for individual cows like the ability to deal with sick cows, or lower culling pressure due to higher involuntary losses associated with less intensive labour investment. Fewer labour units per 100 cows may also be associated with a recently expanded herd size (due to limited financial resources being diverted to herd expansion, and failure to account for increasing labour requirements with increasing herd size), and the lower culling pressure typically associated with expanding herd size is likely to increase the chance of a PI remaining in the herd between seasons.

Herds where the owner reported that more than 20 cows made contact over the fence with neighbours' stock when such contact was possible had higher antibody levels than herds that either reported fewer cows actually making contact or were not able to comment. Care should be taken interpreting this variable because 53% of respondents were not able to comment on this question. It may be an indicator for cow inquisitiveness which appears to vary widely between herds according to stock managers. If so, this may be related to contacts with calves on the same property as well, although this was not asked. It is plausible that herds of inquisitive cows are more likely to interact with neighbours' stock when the opportunity occurs, but it may also relate to the effect of feeding levels and competition for resources on cow behaviour. Since this is a subjective question and relies on a farmer's history of observation and interaction with the herd, it may be the nature of the respondents themselves and their relationship with or attitudes toward their cows that is being measured. Further study including direct observation of herds when grazing adjacent to neighbours stock would help to clarify what role cow behaviour plays for farm to farm transmission of infectious pathogens.

Owner operators or farms with a manager employed had the lowest antibody levels. Higher order share milkers had the next lowest, followed by lower order share milkers with the highest antibody levels. Higher order share milkers own the cows but not the land and are often on fixed term contracts (often 3 seasons) with an incentive to grow the herd and move to a larger farm when their herd outgrows the current location. Lower order share milkers may own some but not all cows in the herd and often plan to rear enough of their own cows to progress to higher order share milking. The effect of ownership structure may be due to bulk stock movements from farm to farm, more frequent and higher number of stock purchases in the past (beyond the scope of the bought cow variable already in the model), and lower culling pressure which may increase the risk of keeping PI or previously infected cows. This may be useful information to inform the type and level of BVD control taken up by various farm systems.

Cow agistment patterns had a significant effect on bulk tank antibody level. Herds that grazed adult cows off-farm for a period in the winter had the highest average bulk tank antibody level as long as the herd was not mixed directly with other stock. Herds that stayed at home had a similar average bulk tank antibody level to herds that mixed their cows with another herd in the winter. In the author's experience, many herds that mixed in the winter were owned by the same person or a close relative and may have been managed by the farmer themselves on a private grazing block. Some of these herds were part of a small cluster of related herds that grazed together and moved stock between herds within the small cluster but had little or no contact with stock from outside the cluster which could be considered a single relatively isolated functional unit. In addition to the risk of exposure from trucking, some grazing locations have beef stock on the same property or close by, and the prevalence of infection is higher in beef herds in New Zealand (Heuer *et al.* 2008). Grazing stock away over the winter period allows pasture reserves to be run lower before drying off, and to build up again before the seasonal calving period so may also be an indicator of farming intensity or stock density.

In New Zealand, many herds use breeding bulls towards the end of the breeding period when cows that had conceived during the preceding period of AI-mating are in the early pregnancy stage when exposure to BVD virus is likely to result in PI fetuses. Many of these breeding bulls come from beef properties or are combined from many sources for bull rearing farms that sell or lease them to dairy farmers. Breeding bulls are the most common introduction onto New Zealand dairy farms and have frequent and close contact to a large number of cows. They can also spread infection venereally as well as other routes in common with cows which should constitute a highly risky and important source of infection. However, no risk variables related to breeding bulls were significant in the models. A possible explanation is a response to awareness campaigns advocating testing and vaccination of breeding bulls which began to affect behaviour in some districts around the time of this study. Bull testing had increasingly been adopted by bull suppliers in response to a subset of their dairy farmers insisting on it. Many bull suppliers had begun testing all bulls before sale due to increasing awareness about the risks and the complexity of testing some bulls but not others. Most testing was done by suppliers prior to sale and dairy farmers were less aware of the risk of introducing BVD virus at the time of this study. Since we relied on information from dairy farmers, there is potential for misclassification of the bull BVD testing status. The number of bulls per herd is relatively low, the prevalence of infection in breeding bulls is fairly low (0.5% is often quoted by New Zealand laboratories from in-house test results), and the intermittent outbreak pattern of BVD virus infections means that PIs sometimes occur in clusters with multiple PIs in a group and many groups with no PIs. It is, therefore, possible that the low-probability, but high impact of this risk factor in combination with widespread control of the risk from breeding bulls meant that this study was simply unable to detect the residual risk from this source. The residual risk from untested bulls may have partly been included in the herd size variable since larger herds typically buy more bulls per season, and possibly the district if testing uptake varied by district which would agree with anecdotes reported to the author at the time of this study.

The other risk factor that was thought to be potentially important was keeping calves from heifers. One reason for this is that PI heifers give birth to PI calves and heifers are more likely to be PI than older cows due to a high PI loss rate (Houe 1993), and a higher risk of culling for poor production (Voges *et al.* 2006). Also, heifers are often grazed away from the home farm (and sometimes transported) during pregnancy and since heifers have had less opportunity to develop immunity than older cows, they may be more likely to be exposed during early pregnancy while still susceptible to infection and therefore be more likely to give birth to a PI than older cows. In New Zealand, some dairy farmers have a management policy of breeding heifers by AI and retaining their calves as replacements. However, the policy of keeping calves from some heifers was not associated with bulk tank BVD status in this study. It is possible that keeping calves from heifers was also associated with another unmeasured variable such as culling pressure (since an increased number of replacements from adding heifers' calves allows for more voluntary culling) that has a roughly opposite effect on BVD status. Given that PI calves are often smaller than their peers and calves from heifers also tend to be smaller, it is possible that PI calves from heifers are often so small that they are rarely chosen as replacements. Also, because PI heifers are often less well-grown and tend to have a higher abortion rate, they may be less likely to conceive and hold to the first round of artificial insemination and it is common for heifers to only receive one AI before the introduction of the bulls so having a policy of keeping calves from some heifers may not substantially increase the risk of keeping a calf from a PI heifer.

The potential role of fomites for introducing BVD virus onto farms such as visiting service personnel (vets, lay-scanners, AI technicians, stock transporters) and vehicles, or tissue movements such as

embryo transfer or artificial insemination, were difficult to assess through the questionnaire. However, such factors are generally considered low risk (Houe 1999), and most visiting service professionals would be expected to follow basic hygiene practices such as washing boots, hands, and equipment. This study, therefore, did not assess the risk of introducing BVD virus to naïve herds through fomites.

The dynamics of bulk milk PCR status change were modelled as the risk that an initially PCR negative herd became newly infected, and the risk that an initially virus positive herd became test negative (i.e. apparently cleared BVD virus infection). While these models are more specific than the other models in terms of distinguishing risk factors associated with maintenance of infection from those associated with new introductions, the number of herds available for analysis was limited, limiting the power of the inferences, and there were insufficient herds per level for many of the potential risk factors to be investigated.

The variables associated with BVD status in decreasing order of significance were herd size; cow purchase without prior BVD virus testing; cow vaccination; district; keeping calves from purchased cows; number making contact over the fence with neighbour's stock when contact is possible; cow ownership; and cow grazing. The factors identified in other studies (herd size, purchase, neighbours, and agistment) are represented in this study with some additional detail such as the risk of keeping calves from purchased cows as well as the animal purchase, and some aspects unique to New Zealand such as cow ownership and district. The effect of other potential risks such as aerial spread and trucking-associated risks may be partially included in the factors already represented in the models. Some factors that are thought to be important such as the risk from bull purchase and the risk from heifers calves were not represented in these models.

Chapter 4: Use of an Enzyme-Linked Immunosorbent Assay for detecting Bovine Viral Diarrhoea virus antibodies in individual cow milk samples

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Published: (Weir *et al.* 2013). *Statement of contribution: Appendix 4*

Introduction

Most dairy herds in New Zealand have at least some cows with antibodies to BVD (Compton and McDougall 2005; Voges 2008). Individual antibody testing of all cows has been advocated as part of a control scheme (Lindberg and Alenius 1999) and may offer benefits in some situations as well as being useful for research purposes. Testing for the level of antibody in the bulk tank milk is gaining popularity in New Zealand for surveillance or screening prior to PCR testing for virus. However, individual cow antibody testing using milk samples is uncommon. Cow-level milk samples collected as part of routine milk production recording (herd testing) offers a cheap and convenient source of samples for investigating BVD.

A number of BVD antibody ELISA kits are available, with the indirect ELISA from IDEXX (Herdchek, IDEXX kit; IDEXX Laboratories, Inc. one IDEXX drive, Westbrook Maine 04092, USA) being used to test bulk and individual milk samples in New Zealand. IDEXX recommends setting the cut-off at sample to positive-control optical density ratio (S/P ratio) of ≥ 0.20 and < 0.30 to define a suspect result and ≥ 0.30 for a positive result for both serum and individual milk samples. Although the original work to set cut-off values is not available, it appears from the IDEXX antibody ELISA validation report[5] that most of the work was done on serum including repeatability (coefficient of variation was 3.54% to 3.95% for intraplate, and 4.6% to 13.7% for interplate), analytical sensitivity (all 4 calves seroconverted between day 9 and day 13 after experimental infection), diagnostic sensitivity (19/19, 100%), and diagnostic specificity (190/190, 100%). Then milk sample analytical sensitivity was assessed using dilution series of 10 milk samples that were positive using an unnamed competitors test (all either positive or suspect without dilution), and milk analytical specificity was assessed using 76 milk samples from a BVD free herd, all of which gave an S/P ratio below 0.1.

Beaudeau et al. (2001c) found that a different BVD antibody ELISA was equally sensitive and specific for milk and serum samples, but that the optimal cut-off value was lower in the milk samples (30% inhibition in milk compared to 50% for serum). Experience with other commercial ELISA kits has shown that their performance with milk samples may vary significantly from serum samples, particularly under New Zealand conditions, and this was confirmed when a small number of animals were incidentally sampled by both milk and serum as part of a larger study relying mostly on milk sampling to establish BVD antibody status. Hence this investigation was conducted to derive more

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appropriate cut points for milk so that milk antibody testing gave a better approximation of the test result when determined in serum.

Materials and methods

Cows

Milk and serum samples were collected from 54 randomly selected cows from two herds in the Waikato region and from 36 cows from one herd in the Taranaki region of New Zealand. One of the Waikato herds had very high (S/P ratio: 1.3) bulk tank milk antibody levels and the other did not (S/P ratio: 0.40). The cows in the Taranaki region were from a herd where BVD virus had been detected by PCR in the bulk tank milk earlier that season. All cows in the Taranaki herd had a milk antibody S/P ratio <0.3 when tested 4 months prior to the paired milk and serum sample collection. Milk and serum samples were collected on the same day for each cow. Samples were collected at 210 ± 39 (mean ± SD; range = 120 to 280, 6 cows with missing information) days in milk, and 56 ± 16 (mean ± SD; range = 11 to 74, 6 cows with missing information) days before dry off. Milk samples were collected into vials containing Bronopol preservative and blood was drawn from the coccygeal vein or artery into an evacuated blood tube which did not contain an anticoagulant. Samples were held at room temperature until they arrived at the laboratory and then held at 4°C overnight and processed the next day. This is the usual treatment for serum samples and milk production recording samples containing Bronopol. Testing occurred within 3 days of sample collection.

None of the cows in this study had been vaccinated against BVD virus.

All procedures involving the experimental use of animals were approved by the Massey University Animal Ethics Committee (Palmerston North, New Zealand).

Laboratory procedures

The S/P ratio was determined in milk and blood using a commercially available ELISA (Herdchek BVD antibody ELISA, IDEXX Laboratories, Westbrook, MA, USA). Testing was conducted by LIC (Hamilton, New Zealand). Blood samples were centrifuged and 25µl of the serum was used for testing. Milk samples were stood over night and the fat manually removed before 100µl of the remaining sample was used for testing. Serum and milk samples from each farm were tested side-by-side (one run by one staff member for each batch of paired samples). All ELISA test plates belonged to the same kit lot. Samples were tested once using the recommended 90-minute incubation protocol.

Statistical Analysis

The relationship between the paired serum and milk S/P values was defined using a linear regression model with milk value as the outcome and serum value as the explanatory variable so that new milk cut-off values could be derived. Higher order models were tested for significance and the best fit model was accepted. The prediction equation given by the coefficients was used to convert the serum cut-point values (0.20, 0.30) to equivalent milk values (0.08, and 0.13). The new cut-off values were used to categorise the milk test results into negative, suspect, and positive. These were then compared to the categories of the paired serum samples using the Fleiss-Cohen weighted Kappa test (Cohen 1968) to assess the change in agreement between the newly categorised milk samples and the paired serum samples, and sensitivity and specificity were reported relative to serum ELISA status excluding uncertain (suspect) results. In addition, Bland-Altman plots and sensitivity and specificity estimates with serum values as a reference were used to evaluate the new versus old cut-off values.

The process of deriving a conversion equation and using it to adjust cut-off values and categorise milk status was then repeated 90 times in a jackknife procedure where an equation and resulting cut-off

values were determined with a sample pair excluded and the resulting cut-off values used to categorise the excluded sample. This process was repeated for all pairs in the dataset, thus each pair was used as an independent sample and tested against the cut-off value generated from the remaining samples with that pair excluded. The Jack-knife procedure gives an indication of the amount of variation that can be expected when applying the calculated estimates to external data assuming independence between cows. The range of estimated cut-off values is reported as the range encompassing 95% of values in this dataset. Days in milk, days to drying off, and age were tested for confounding by adding each one at a time into the final model and *p*-values reported.

Analyses were conducted using R 2.15.0 (R Core Team 2014).

Results

The model to predict a milk value from a serum value without an intercept (intercept $p=0.53$ so the model was run without an intercept term) included serum value and serum value squared (Figure 3), both highly significant ($p \leq 0.01$), and yielded the equation ($R^2 = 0.78$):

$$\text{milk S/P} = 0.385 \times \text{serum S/P} + 0.180 \times \text{serum S/P}^2$$

Only one cow with a 2.0 S/P ratio in serum and in milk approached 0.5 units of Cooks distance (Cook 1977) in the residual analysis. It was not sufficiently influential to justify exclusion. From this equation, the adjusted milk cut-off value between negative and suspect, and suspect and positive were defined as ≥ 0.08, and ≥ 0.13, respectively. The agreement between serum status and milk status using the previously recommended cut-off values for both is presented in Table 10, and the agreement between serum status and milk status using the adjusted cut-off values for milk is presented in Table 11. The Kappa values indicate a substantial improvement (0.58 to 0.82) of the ability of milk testing to approximate serum status as defined by the IDEXX ELISA.

An alternative method for assessing agreement using continuous measures is the Bland-Altman plot. Figure 4a presents the plot for the raw S/P ratios showing that there is a clear difference in scale between milk and serum resulting in a poor agreement. Figure 4b presents the plot with serum converted to the milk scale using the linear regression equation which shows a better fit between the two tests indicating that the equation accounted for a systematic difference between the sample types.

The jackknife procedure resulted in 95% of the lower cut-off value estimates for suspect samples between ≥ 0.080 and ≥ 0.087 (minimum 0.079, maximum 0.091), and 95% of the upper cut-off value estimates for positive samples between ≥ 0.126 and ≥ 0.136 (minimum 0.124, maximum 0.141). The median cut-off value estimates were ≥ 0.084, and ≥ 0.132.

The adjusted cut-off value categories resulted in a relative sensitivity of 100% (61 / 61+0), and a relative specificity of 94% (18 / 18+1) for milk relative to serum ELISA status, compared to 86% (44 / 44+7), and 100% (18 / 18+0) respectively for unadjusted milk status relative to serum ELISA status. Moreover, the change to the new cut-off reduced the rate of suspect results based on testing milk from 16% (14/90) to 2% (2/90).

Days in milk ($p=0.33$), days to dry off ($p=0.78$), and age ($p=0.78$) were not significant when tested in the regression model one at a time which indicates that none of these variables had an important influence on the relationship between serum and milk values in this data set.

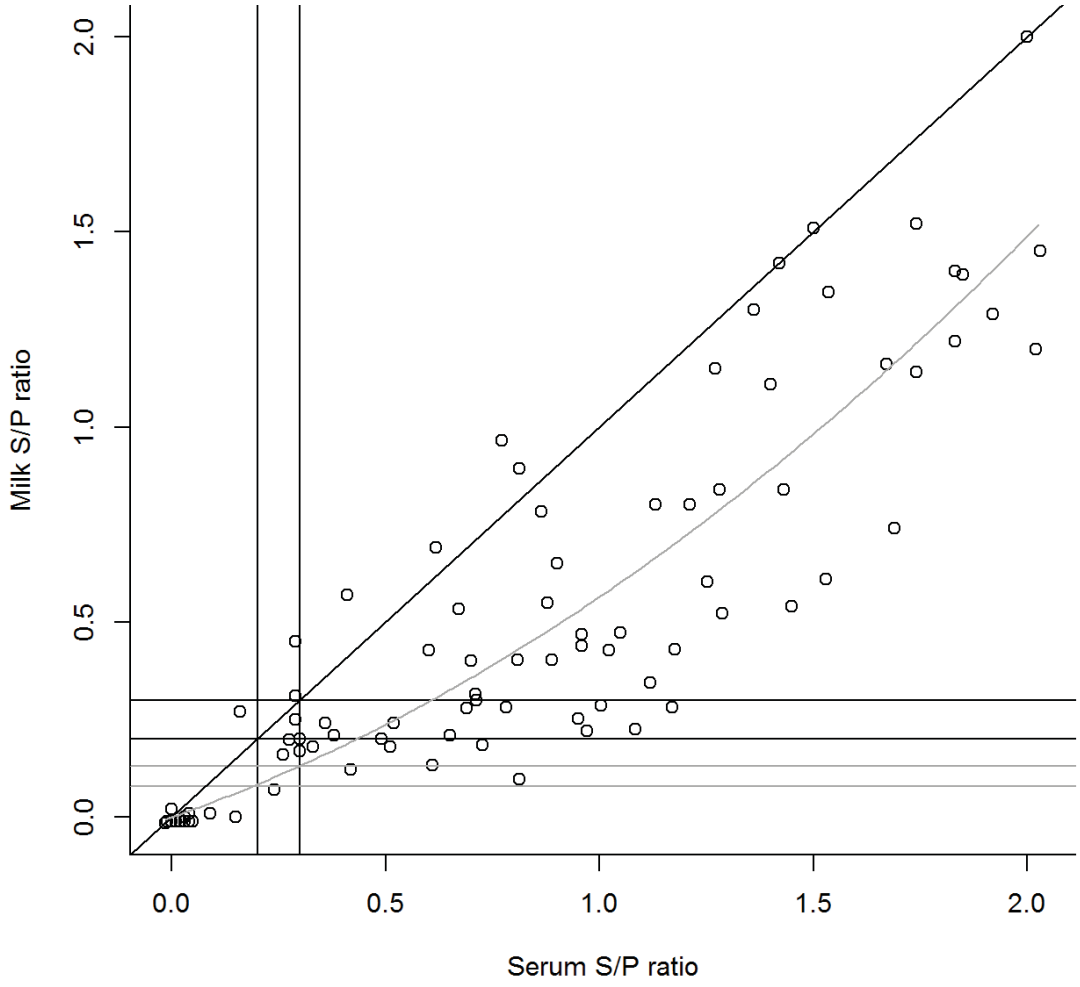

Figure 3: Plot of IDEXX BVD milk antibody sample to positive-control ratio (S/P ratio) by serum S/P ratio with recommended cut-off values (dark horizontal and vertical lines at 0.2 and 0.3) and adjusted cut-off values (grey horizontal lines at 0.08 and 0.13), the equivalency line (diagonal black 1:1 – implied by using the same cut-off values for the different sample types), and the best fit regression line (grey curve, adjusted $R^2 = 0.89$).

Table 10: Comparison of IDEXX BVD antibody ELISA sample to positive-control optical density ratio category of paired serum and milk samples using previously recommended cut-off values

Milk status	Serum status Negative (< 0.2)	Suspect (≥ 0.2, < 0.3)	Positive (≥ 0.3)	Total
Negative (< 0.2)	18	5	7	30
Suspect (≥ 0.2, < 0.3)	1	1	12	14
Positive (≥ 0.3)	0	2	44	46
Total	19	8	63	90

The Fleiss-Cohen weighted Kappa test for agreement between the paired tests gives a value of 0.58 +/- 0.131 indicating a substantial difference between milk and serum test results on the same cow using the previously recommended cut-off values. The McNemar's Chi-squared test for difference $p<0.001$ which means there was a highly significant difference between the 2 tests. There are many (7/30) false negatives for milk.

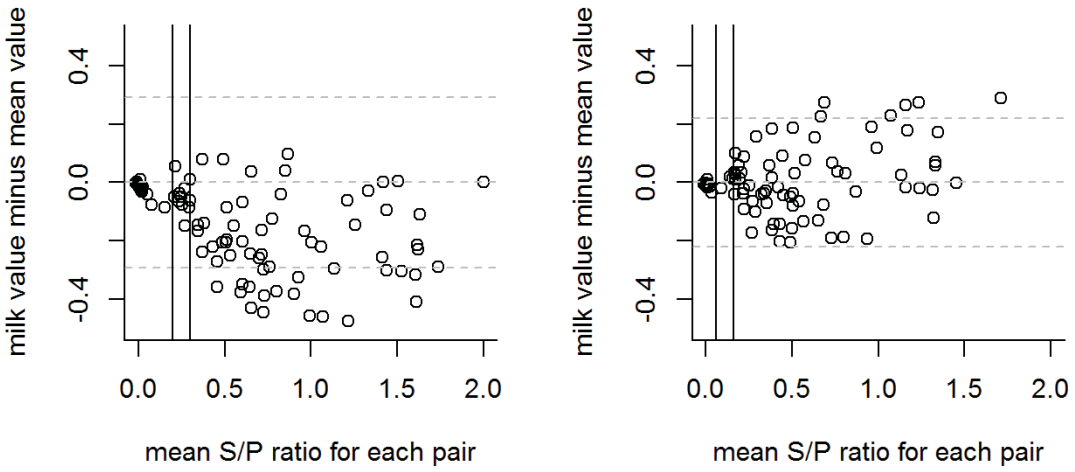

Figure 4: Bland-Altman plot showing the relationship between paired milk and serum sample to positive-control ratio values for raw data (a), and with serum values rescaled to the milk value scale using the equation from a linear regression model to account for the systematic difference (b). Serum was converted to the milk value scale because that was the method used to derive new milk cut-off values. The upper and lower dotted lines are 1.96 standard deviations away from zero mean value difference, and the vertical lines divide the mean values into negative, suspect, and positive status (left to right) based on the previously recommended cut-off values (a), and the adjusted cut-off values (b). Plot (a) shows that milk values tend to be lower than serum values greater than zero. Plot (b) shows that applying the conversion accounts for this systematic difference and brings them in line with each other with few values falling outside the 95% interval range.

Table 11: Comparison of IDEXX BVD antibody ELISA sample to positive-control ratio category of paired serum and milk samples using new, adjusted cut-off values for the milk samples

	Serum status			
Milk status	Negative (< 0.2)	Suspect (≥ 0.2, < 0.3)	Positive (≥ 0.3)	Total
Negative (< 0.08)	18	1	0	19
Suspect (≥ 0.08, < 0.13)	0	0	2	2
Positive (≥ 0.13)	1	7	61	69
Total	19	8	63	90

The Fleiss-Cohen weighted Kappa test for agreement between the tests gives a value of 0.82 +/- 0.105 which is much improved compared to using the previously recommended cut-off values (see Table 10). The McNemar's Chi-squared test for difference p=0.19 which means there was no significant difference between the 2 tests using these new cut-off values.

Discussion

The application of the newly defined cut-off values resulted in a substantially higher level of agreement between serum and milk antibody BVD ELISA test results than the previously recommended cut points. The size of the increase in agreement shows that there was a systematic difference between serum and milk and that the proposed adjustment was able to account for this difference. Moreover, the new cut-off increased the relative sensitivity of detecting BVD antibody while maintaining relative specificity.

Another benefit was a substantial reduction in the rate of suspect results. Using the adjusted suspect range for milk samples resulted in all serum suspect cows being either positive (7) or negative (1) on milk. With a smaller absolute range (0.05 units) for suspect results, test variability may cause an increased rate of misclassification of low positive or negative cows as negative or low positive respectively. This cannot be avoided for milk samples without inflating the suspect range which would also increase the rate at which positive or negative cows are classified as suspect which is undesirable. It may be appropriate to arbitrarily inflate the suspect range in some situations depending on which type of error is least desirable.

The IDEXX antibody ELISA on serum was the reference test in this study but the model was set up to adjust the cut-off values to the milk scale so that future milk samples would not require a conversion before a status could be assigned. It is uncommon for serum antibody levels to exceed 2.0 S/P ratio units using the IDEXX ELISA so the observed serum values covered the typical range expected of field samples. There were 19 (21%) cows that were negative on serum. This may be different in some other herds but could only introduce a substantial bias in the conversion formula if there was a large difference in the relationship between serum and milk antibody level over the range of possible results that was not captured by the regression model. Since serum negative cows are clustered around zero on serum and on milk, a larger number of negative cows would have little effect on the model estimates.

Although it would be of interest to compare the milk ELISA to a gold standard for serum status, further validating the accuracy of the test or reassessing the cut-off values in serum was beyond the scope of this study. Hence, the gold standard for this study was the same IDEXX ELISA applied to serum samples as the one applied to milk samples. A validation report detailing the work done to validate the test is available on request from IDEXX. As long as the serum ELISA is the accepted standard test for practical purposes, the adjusted cut-off values for the milk ELISA provide a more accurate representation of serum ELISA results.

There was excellent agreement between milk and serum using the adjusted cut-off values but serum remains the preferred sample type, especially where low positive antibody levels are likely to occur. There are times, however, when blood sampling is not feasible or desirable such as when large numbers of samples must be collected in a short period of time or where access to stock is limited to milking time. For these situations, the adjusted cut-off values for milk samples will be more reliable than the previously recommended ones.

Variation due to test performance (repeatability) should only be an issue for samples with antibody levels close to a cut-off value and will increase the chance of disagreements between the tests but should not introduce bias unless there was a strong plate-level effect, affecting all 3 sample sets in the same way (milk and serum pairs were not run on the same plate). Since there were few disagreements, it is unlikely that variation due to within-plate test performance had a substantial influence on the cut-off value adjustment. Since there were a small number of plates used and paired samples were run on different plates, it is possible that the inter-plate variability of the test had a small effect on the reported adjustment.

An interpretation of antibody levels in early or late lactation should be undertaken with care due to elevated antibody levels in colostrum or increased proportion of milk solids with low production in late lactation. However, as no samples were collected from < 120 days in milk, colostrum would not have affected these results. A few of the samples in this study may have been affected by a higher concentration of solids in late lactation since some of them came from cows that were close to being dried off, but the agreement between the tests and the lack of statistical significance of days to dry off indicate that it was not an important factor in this study

There was only 1 (1.1%) milk sample with a completely different status from the paired serum sample using the new cut-off values (serum negative, milk positive) whereas there were 7 (7.8%) that differed before adjustment (all serum positive, milk negative). All but one of the suspect results for both sample types were either suspect or positive on the other sample type (Table 11). While a suspect result could be treated as a positive, a more defensible strategy may be to repeat the test several weeks later so that a recently infected cow may have an increased BVD antibody concentration resulting in a clearly positive result.

These results show a similar pattern to Beaudeau et al. (2001c) in that the milk antibody levels tended to be lower than serum antibody levels. The best fit relationship had a serum squared term indicating that the antibody concentrations in milk increased more slowly than in serum at lower levels of antibody. When a straight line was fitted (not shown), the milk S/P ratio was about two-thirds (0.63) of the serum S/P ratio. Using the same cut-off value as serum returned a large proportion (7/30, 23%) of false negative results (Table 10). After accounting for the relationship using the new cut-off values, there were very few disagreements about negative serum results, and all of the serum positive cows were either positive or suspect on milk (Table 11).

When using milk samples collected during milk production recording, there is a risk that some milk may still be present in the collection equipment from the preceding cow. The volume of this carry over milk is likely small compared to the total milk volume so is unlikely to substantially affect the test result. However, it is a potential limitation of the sampling method and care should be taken during milk collection to avoid it. It is possible that the single cow in our data with a negative serum and positive milk result using the new cut-off value was due to such a carry-over effect but it would take many more serum negative duplicate samples to estimate the likelihood of this kind of error and

the likelihood would be affected by the prevalence of antibody positive cows and the level of antibodies of the positive cows in the herd.

Increasing the number of samples assessed may have increased the precision of the adjustment, and sampling from more than three herds may have increased the external validity of the study, particularly including herds with different prevalence of exposure. We evaluated the potential external validity using the jackknife analysis. This resulted in very little variation around the selected new cut-off values, hence the cow-level variation in the relationship between serum and milk values appeared to be relatively small. Although factors like production level or nutrition might have some effect on milk antibody level without affecting serum levels as much and therefore violate the assumption of independence between cows for the jackknife analysis, it is likely that individual variation would be greater than any potential herd effect on the relationship between milk and serum. Thus we believe the external validity of the new cut-off values is reasonably high and that inference about BVD antibody from future milk ELISA testing would benefit from using them.

In conclusion, the authors suggest using the adjusted cut-off values (≥ 0.08 between negative and suspect, and ≥ 0.13 between suspect and positive) for individual milk samples tested with the IDEXX antibody ELISA instead of the previously suggested values when milk testing is required instead of serum testing.

Chapter 5: Impact of transient infection with Bovine Viral Diarrhoea (BVD) virus on reproductive performance and health of dairy cows

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Statement of contribution: Appendix 5

Introduction

Infection of susceptible cows with Bovine Viral Diarrhoea (BVD) virus prior to or shortly after conception can adversely affect fertility (McGowan *et al.* 1993a) due to direct effects on ovarian tissue (Ssentongo *et al.* 1980; Fray *et al.* 2000a). If infection occurs during pregnancy, the virus may cross the placenta and infect the fetus (Fray *et al.* 2000b; Stokstad *et al.* 2003). As long as the fetus is not immunocompetent (up to 125 days of pregnancy), infection can cause early embryonic loss, abortion or life-long persistent infection (PI) of the fetus. Once immunocompetent, the fetus will seroconvert but infection may still lead to abortion or congenital malformation (Grooms 2006). The response to infection depends on host immune response, environmental stress and virus strain and this is reflected in variation in outcomes reported amongst studies (Houe 1999).

Immune suppression has been demonstrated in adult cattle following exposure to BVD virus. This occurs at least partly due to infection of cells critical to the immune response (Chase *et al.* 2004) and leukopenia, lymphopenia, and thrombocytopenia (Lanyon *et al.* 2014). Niskanen et al. (1995) found that newly infected herds (low bulk tank milk (BTM) antibody one year and high BTM antibody the next year) had higher incidence rates of mastitis (OR: 1.8), miscellaneous diseases (OR 2.8), and retained placenta (OR: 2.8) compared to herds that maintained low BTM antibody levels. In a similar study, Waage (2000) observed an increase of 7% in the incidence of clinical mastitis in herds with a new BVD virus infection. Moreover, herds with a high incidence of production diseases were more likely to have BVD virus positive bulk milk than bulk milk negative herds (Kozasa *et al.* 2005).

Most dairy farms in New Zealand are highly seasonal, pasture-based systems with nearly all cows calving between July and October to match the cows' nutrient demands with pasture growth. Herds have a planned start of mating date (PSM) before which no cows are mated, and a single date 9 to 18 weeks later (most herds 11 to 14 weeks) when bulls are removed and mating ceases. Bulls are usually introduced after 4 to 6 weeks of artificial insemination. Cows remaining non-pregnant at the end of the mating period are culled by the end of the milking season apart from a small proportion in some herds which are "carried over" in a non-pregnant state to be mated again in the following season. Most cows in a herd are dried off on the same day irrespective of lactation length or production level. No cows are milked subsequently until calving begins in the next season. Some cows may be dried off early, however, depending on body condition and pasture availability. Because of this seasonal reproduction cycle, the infection dynamics and impact of BVD virus may be different than in year-round calving management systems with little seasonality. For example, introduction of infection after the last AI fetus is 125 days will not result in any PI replacements, while the introduction of infection into a mostly naïve herd late in the mating period could result in many PI replacements born in the subsequent season. Most studies, including field studies in year-round systems (Rufenacht *et al.*

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2001), investigated the effect of infection at known stages of pregnancy. In an observational study such as this, some cows will be infected prior to their first mating, and others long after conception. However, this study is interested in the average effect over this time which describes the total expect impact of transient infection in seasonal calving herds.

This study aimed to investigate the infection dynamics of BVD virus and quantify the effect of transient infection on reproduction and health from the planned start of mating through to the final individual cow production test (herd test) in lactating New Zealand dairy cows.

Materials and methods

This is a prospective cohort study with the cow as the unit of interest.

Herds

This study targeted dairy herds with active BVD virus infection. In Spring 2007, BTM samples from 206 herds were tested for BVD virus 2 to 4 weeks (median 13 days, 95% range: 21d before to 3d after) before the PSM using a real-time Polymerase Chain Reaction (PCR) test (Testlink, Livestock Improvement Corporation Ltd, Hamilton, New Zealand). This included 13 PCR positive herds, 2 of which were vaccinated against BVD virus so were not eligible for enrolment. Of the 11 remaining herds, 10 were enrolled for cow-level investigation. All but 2 enrolled herds had vaccinated calves with an inactivated BVD vaccine since at least 2005, and 2 herds also vaccinated yearlings pre-mating, but none vaccinated the milking herd. Antibody titres from inactivated vaccines are detectible for less than a year and any protection provided would not be expected to persist beyond entry to the milking herd.

Testing

An individual cow composite milk sample was collected via an in-line meter (either "Waikato meter", or "WB Auto Sampler", both from Tru-Test Ltd, Auckland, New Zealand) from all cows at an afternoon milking one to two weeks after the PSM. The antibody titre was determined by an indirect antibody ELISA (Herdchek, IDEXX kit; IDEXX Laboratories, Inc. one IDEXX drive, Westbrook Maine 04092, USA), using the overnight incubation protocol. All testing was conducted by a commercial laboratory (Genemark Animal Health, Hamilton, New Zealand). Samples with an antibody titre <0.30 sample to positive (S/P) ratio were also tested for virus. Antibody starts being detectable 2 to 3 weeks after infection (Fredriksen *et al.* 1999; Houe *et al.* 2006), hence a test positive result indicated exposure before PSM. Antibody testing was repeated for all cows with an initial milk ELISA S/P ratio value of < 0.3 (the IDEXX recommended cut-off value for a positive result) four to five months later at a subsequent milk production recording. Any cows that missed this collection or aborted after this sample were blood sampled for antibody testing where possible. Cows were defined antibody positive where the S/P was ≥0.13, suspect if ≥0.08 and <0.13, and negative where <0.08 which is equivalent to the recommended serum cut-off values of 0.2 & 0.3 (Weir *et al.* 2013). Cows without a milk sample had serum samples collected and these were categorised using the standard serum cut off values. Finally, cows were categorised as follows:

- "immune": initially antibody positive, or initially suspect and suspect or positive at second test with milk antibody level increased by <0.05 units;
- "naïve": antibody negative at the second test, or antibody negative at the first test and suspect at the second test but titre increased by <0.05 units;
- "transiently infected (TI)": antibody negative or suspect at the start, and increased by >0.05 units to become suspect or positive.

For evaluating the effect of transient infection, immune and naive cows were combined and defined as control cows because neither experienced a new infection. The threshold for status change of 0.05 units was taken from the width of the suspect range (0.13-0.08 units).

Data

Cow calving date, age and artificial insemination dates (recorded by technicians at the time of insemination) were recovered from a central database (Livestock Improvement Corporation ltd., Corner Ruakura and Morrinsville Roads, Newstead, Hamilton, New Zealand). Bull mating dates were recorded by herd managers to the best of their ability and entered manually. Conception was determined by ultrasound scanning 12 to 14 weeks after the planned start of mating, again 6 weeks after that, and a third time at least 6 weeks after the end of mating if required (where the total mating period was >14 weeks) to accurately define the date of conception for all pregnancies. Recorded conception dates were compared to calving dates in the subsequent season and a small number were adjusted with this additional information to maximise conception date accuracy. If a full-term calving date was not within 12 days of the predicted calving date, and there was a recorded mating date that indicated a predicted calving date that was within 12 days of the actual calving date, then conception date was set to this mating date instead of the one selected at the time of scanning.

Herd managers were asked to keep detailed records of each observed illness event, and record treatments and the dates that milk from treated cows were withheld. Recording treatment and withholding times were conditions of supply and farmers were obliged to record them on standard recording sheets. These data were entered manually into the database. The drugs that farmers had in store for animal treatment were audited at the time of enrolment and farmers were asked to retain all treatment packages in a designated location. The disposed of packages were audited at the end of the investigation period along with all unused drugs. This “trash audit” information was combined with vet clinic sale records (the exclusive source of additional products) and compared to treatment records to ensure accurate recording. Any apparently missing treatment records were reconciled with records held by farm staff and updated as appropriate.

Analysis

Data were entered and stored in a relational database (Microsoft Access), validated using routine procedures (missing values, min/max, and outlier analyses), and analysed using R 3.1.2 (R Core Team 2014). Outcomes of interest of this study were the proportions of the herd (i) remaining naïve, (ii) immune at the start (iii) seroconverting during the study period (TI), (iv) finally pregnant, (v) aborting, the intervals of (vi) PSM to conception, and (vii) conception to artificial insemination, (viii) drug plus discarded milk total cost of illness.

PI animals were excluded from all analyses. Data from cows that seroconverted during the observation period (TI) were compared to control cows. Potential confounding factors including herd, age, calving date, and breed were tested and retained in multivariable models if significant at $p \leq 0.05$.

To appreciate the cost of non-pregnant days, a standardised daily production value was used to convert days to a dollar value. The New Zealand dollar was worth approximately US$0.79 during the observation period. The average production for New Zealand cows in 2007 was 1.33 kg milk solids (i.e. fat kg + protein kg) per day[4]. The value of milk in the 2007 season for Fonterra suppliers (all

[4] http://www.lic.co.nz/pdf/DAIRY_STATISTICS_07-08.pdf

herds in this study) was $7.66 per kg milk solids[5]. The average value of milk production was, therefore, NZ$10.19 per cow per day (US$8.05).

The number of cows in each exposure category and herd and the number of cows in each exposure category and parity group were summarised in tables. Differences in initial exposure level and seroconversion rates between parity groups were tested for significance using GEE models accounting for herd with parity group as the only explanatory variable. The number of potentially effective contacts (contacts that would cause a transient infection if the cow was susceptible) per PI per day were calculated assuming random mixing with all cows having an equal chance of contact with a herd mate. This ignored any segregation into management mobs and social grouping factors. The proportion of initially antibody negative cows seroconverting during the study period was assumed to be the same as the proportion of the herd making potentially effective contacts with the PI over that period. The proportion of the herd to have made contact with the PI, therefore, would be 1 – (1 – daily probability of contact per cow)^{days}. The daily probability of contact per cow was therefore 1 – (1 – proportion of the herd to have made contact with the PI)$^{1/days}$. The number of effective contacts the PI makes per day then would be the daily probability of a cow making contact multiplied by the herd size.

Reproduction

The time from PSM to conception was modelled using a marginal accelerated failure time (AFT) model with herd as a cluster variable. The AFT model was used to calculate the predicted number of days delayed conception for transiently infected cows at the mean calving to PSM interval. The number of days conception was delayed was then converted to a dollar value using the standardised production value of NZ$10.19.

First service conception rate, conception to artificial insemination (AI), conception in the first 6 weeks of mating, and abortion risk through to drying off were modelled using a logistic model accounting for interval (d) from calving to PSM in the first 3 models. The correlation of cows in herds was modelled by the generalised estimating equations (GEE) algorithm in the R-package 'geepack' (Højsgaard *et al.* 2006). The cost of BVD virus associated reductions in first service conception rate, 6-week pregnancy rate, and abortion risk were captured by the PSM to conception model and the pregnancy model, but the cost of lost conceptions to AI due to delayed conception and later natural mating was a separate cost. The average value for the difference between AI bred calves compared to non-AI bred calves was $141 after accounting for 5% calf loss, 50% sex ratio, and increased beef breed bobby calf value (weighted average of herds using beef breed bulls for mating)[6].

The proportion of cows finally pregnant was modelled using logistic GEE regression. Odds ratios (OR) were converted to relative risk using the method described by (Zhang and Yu 1998) because pregnancy was not rare. The average effect of transient infection on the proportion pregnant was estimated by taking the difference between TI and control cows of the weighted average proportion pregnant predicted by the model. The weights for these weighted averages were the number of cows with each combination of predictor levels. The mean planned start of calving to calving date interval was used for the prediction. This predicted difference in proportion pregnant was converted to a dollar value by multiplying the difference by $951, the difference between the value of replacement heifer

[5] http://www.interest.co.nz/rural-data/dairy-industry-payout-history
[6] Fertility economic model of the New Zealand Animal Evaluation Limited (http://www.dairynz.co.nz/media/928747/Fertility-Economic-Model.pdf)

and the value of a culled cow taken from New Zealand animal evaluation models[7]. Log-logistic, log-normal, and Weibull distributions were tested for the AFT models and the distribution which gave the smallest AIC was used. The linearity of continuous variables was tested using polynomials and higher level polynomials were retained if appropriate.

Disease

Disease cost was calculated as cost of drugs used plus the recorded number of days of milk discarded multiplied by the standardised daily milk cost. There was a large excess of zero cost since many cows had no disease during the observation period (89.2%), with a highly overdispersed, right skewed count of cost for cows experiencing disease events (mean $126, variance $6,842). Disease cost was therefore modelled using a hurdle model (Zeileis *et al.* 2008) with a logistic component for "any disease", and a negative binomial component for the cost of disease where disease occurred. The weighted average predicted cost per cow from the model (weighted by the number of cows with each combination of variables and using a predict function on the model) were then calculated for TI cows and control cows to calculate the difference in total cost due to transient infection.

The ratio TI:control of the predicted costs was used to estimate the marginal increase in disease risk during an assumed 28 days of immune suppression because the effect of transient infection was likely to have a limited duration and would be obscured by the base disease risk over the much longer observation period. The observed ratio depended on the length of the observation period. The marginal increase in disease risk over the full time at risk (the ratio – 1) was divided by the proportion of the average time at risk that the 28 days made up (28 / average time at risk) to get the total marginal increase in disease risk due to transient infection required to get the observed rate. The relative risk of disease during this period was, therefore, the calculated marginal increase plus 1 for the base rate.

The effect of transient infection on at least one case of mastitis (binomial), number of cases of mastitis (negative binomial), count of days ill due to mastitis (negative binomial), at least one case of lameness (binomial), and number of cases of lameness (negative binomial) was investigated with regression models (logistic or negative binomial as appropriate) accounting for parity and herd. Time at risk was also accounted for in the negative binomial models. The estimate, standard error, p-value, and odds ratio or incidence rate ratio for transient infection status from each model is reported.

Results

There were 3,806 enrolled cows, of which 964 were sampled a second time, and 13 cows' BVD status could not be classified because they were initially antibody negative or suspect and were removed before a second test was undertaken. The number of cows in each BVD antibody category by herd is presented in Table 12. In the herds where a PI was not detected amongst the milking cows, 31.3% were susceptible and 31.7% of these seroconverted. In the herds where a PI was detected, only 3.8% of the cows were susceptible but 82.4% of them seroconverted during the study.

The mean number of days between first and last antibody test was 125 days. The daily probability of a cow making effective contact with a PI, in herds where a PI was present, was therefore $1 - (1 - 82.4\%)^{1/125} = 0.0138$. Two of the herds had 100% seroconversion rate so 125 days was greater than the actual time at risk until the last cow seroconverted in those herds, thus the daily probability of contact was underestimated in these herds. The average number of potentially effective contacts per

[7] From the live weight, and the longevity residual survival economic models of New Zealand Animal Evaluation Limited: http://www.dairynz.co.nz/media/928750/Liveweight-Economic-Model.pdf, and http://www.dairynz.co.nz/media/928753/Longevity_Residual-Survival-Economic-Model.pdf

PI was therefore 0.0138 x 2,684 cows / 8 PIs = 4.6 cows per day. Daily contact rates and contacts per PI based on the seroconversion rate for herds 4-8 (herds with a PI but less than 100% seroconversion rate) were 0.0127 and 3.8 cows, and for herds 1-3 (herds without a milking PI) assuming 1 non-milking PI per herd were 0.003 and 0.48 cows. The time at risk required for the herds without a lactating PI (herds 1-3) to get a similar rate of effective contacts per cow per day as the herds with a lactating PI (herds 4-10) was 13 days of exposure. If contact was possible on only one day, for example, if the seroconversions were due to a single cross-fence contact event, and no subsequent transmission occurred then an average of 50 potentially effective contacts were required on that day in these herds (14% of the herd).

Table 12: Number of cows, number of animals confirmed as persistently infected BVD virus carriers (PI), number and percentage of initially susceptible cows, number of cows defined as BVD virus immune, number of naïve, and number of transiently infected (TI) cows with percent of susceptible and percent of herd amongst 10 dairy herds that were test positive for the presence of BVD virus in bulk tank milk

Herd	Cows[1]	PI	Susceptible[2]	Immune[3]	Naïve[4]	TI[5]	TI[5]	TI[5]
	n	n	n (% of herd)	n	n	n	% of [2]	% of [1]
1	314	0	170 (54.1%)	144	140	30	17.6%	9.6%
2	468	0	108 (23.1%)	360	74	34	31.5%	7.3%
3	327	0	69 (21.1%)	258	23	46	66.7%	14.1%
4	255	1	15 (5.9%)	239	4	11	73.3%	4.3%
5	285	1	9 (3.2%)	275	2	7	77.8%	2.5%
6	241	1	9 (3.7%)	231	2	7	77.8%	2.9%
7	342	1	11 (3.2%)	330	2	9	81.8%	2.6%
8	948	1	45 (4.7%)	902	8	37	82.2%	3.9%
9	365	2	4 (1.1%)	359	0	4	100.0%	1.1%
10	248	1	9 (3.6%)	238	0	9	100.0%	3.6%
All	3,793	8	449 (11.8%)	3,336	255	194	43.2%	5.1%

[1] Number of cows by herd
[2] Susceptible to infection at the first test: Naïve or TI cows
[3] Immune: initially antibody positive, or initially suspect and suspect or positive at second test with milk antibody level increased by <0.05 units
[4] Naïve: antibody negative at the second test, or antibody negative at the first test and suspect at the second test but increased by <0.05 units
[5] Transiently infected (TI): antibody negative or suspect at the start, and increased by >0.05 units into at least the suspect category
There were 8, 1, 1, 0, 0, 0, 1, 1, 0, 1 initially susceptible cows lost to follow-up from herds 1 through 10 respectively (excluded from the table).

The median calving date relative to the planned start of calving was 19 days (95% range: -6 days to +68 days). The median calving date was 14, 18, 14, 16, 21, 28, 20, 21, 16, and 21 days after the planned start of calving for herds 1 through 10 respectively. There were 122 (38%) cows with no recorded calving date in herd 1, but the other herds had 9, 0, 0, 0, 1, 0, 4, 1, 0 cows with no recorded calving date for herds 2 through 10 respectively.

The PI cows were parity 1 (one each from herd 7, 9, and 10), parity 2 (one each from herd 4, 8, and 9), or parity 3 (one each from herd 5 and 6), and had antigen ELISA S/P ratios ranging from 3.5 to 4.2 at the first test and 4.1 to 4.4 at the final test. Only 2 of the 8 PIs survived to calve in the 2008 season as 3 were culled (1 for failing to conceive, 1 for aborting, and 1 for low production), and 3 died. One of the PI cows that survived to calve in the 2008 season had a dead calf and the other had a bull calf that was removed at 4 days of age.

There were 4 cows with antigen and no antibody (i.e. S/P <0.13) at the first sample, and no antigen but antibody present at the final sample indicating that they were transiently infected at the time of first sampling. The antigen levels were 4.4, 4.0, 3.7, and 1.4 units compared to a mean 4.0 units for the confirmed PI cows. If the probability of a cow making effective contact with a PI in the herds with a PI (herds 4-10) was 0.0138, then the number of transiently infected cows per day around the start of mating would have been 0.0138 x 102 initially susceptible cows = 1.4 transiently infected cows per 24 hours. The average duration of shedding that would result in 4 transiently cows detected would then be 4 detected / 1.4 per 24 hours = 2.9 days.

The number of cows with each exposure status by parity group is shown in Table 13. Parity 1 cows were not more likely to be susceptible than parity 2 cows, but parity 1 and 2 cows were more likely to be susceptible at the first test than older cows. Initially susceptible parity 2 cows were the least likely to seroconvert by the final test followed by parity 1 cows, then parity 4 or more cows, and parity 3 or 4 cows were the most likely to seroconvert. However, the chance of parity 4 or more cows seroconverting was not significantly different to parity 1, or parity 3 or 4 cows. The difference in seroconversion rate between parity group indicates that contact was not completely random. There were only 2 initially susceptible parity 1 cows in herds with a parity 1 PI (both in herd 9). There were 1, 2, and 14 initially susceptible parity 2 cows in herds 9, 4, and 8 respectively, with a parity 2 PI, and there were 3, and 6 initially susceptible parity 3 cows in herds 5 and 6 respectively, with a parity 3 PI, so despite being in contact for at least 2 years and up to 6 years, some of these peers apparently remained susceptible, though most (20 / 26) did seroconvert during the observation period. Herds without a parity 1 PI had 1, 1, 2, and 11 initially susceptible parity 1 cows in herds 4, 5, 6, and 8 respectively, so parity 1 cows did not appear to represent a substantial pool of susceptible cows for these herds.

Table 13: Number of cows, number of animals confirmed as BVD virus carriers (PI), number and percentage of initially susceptible cows, number of cows defined as BVD virus immune, number of naïve, and number of transiently infected (TI) cows with percent of susceptible and percent of herd by parity group

Parity	Cows[1]	PI	Susceptible[2]	Immune[3]	Naïve[4]	TI[5]	TI[5]	TI[5]
	n	n	n (% of cohort)	n	n	n	(% of [2])	(% of [1])
1	574	3	108 (18.8%[a])	466	70	38	35.2%[a]	6.6%
2	651	3	146 (22.4%[a])	505	97	49	33.6%[b]	7.5%
3 or 4	987	2	86 (8.7%[b])	901	37	49	57.0%[c]	5.0%
> 4	1557	0	109 (7.0%[c])	1448	51	58	53.2%[ac]	3.7%

Values with different superscript letters within a column are significantly different at p≤0.05 in a GEE model accounting for herd clusters ("exchangeable" correlation structure) with parity group as the only explanatory variable.

[1] Number of cows in each parity group

[2] Susceptible to infection at the first test: Naïve[4] or TI[5] cows

[3] Immune: initially antibody positive, or initially suspect and suspect or positive at second test with milk antibody level increased by <0.05 units

[4] Naïve: antibody negative at the second test, or antibody negative at the first test and suspect at the second test but increased by <0.05 units

[5] Transiently infected (TI): antibody negative or suspect at the start, and increased by >0.05 units into at least the suspect category

There were 2, 1, 3, and 6 initially susceptible cows lost to follow-up with parity 1, 2, 3 or 4, and 5 or more respectively (excluded from the table).

Reproduction

Planned start of mating (PSM) to conception

The simple survival curves of TI cows compared to control cows are presented in Figure 5.

Results of the AFT model of planned start of mating (PSM) to conception are presented in Table 14. The weighted average number of days from PSM to conception for cows that calved 60 days (the mean) before the start of mating was 27.0 days for TI cows, and 23.8 days for control cows (p = 0.157). The average delay attributable to transient infection was therefore 3.2 days delayed conception equivalent to a cost of 3.2 x \$10.19 = \$32.61 due to lost milk production in the subsequent season.

Final pregnancy status

The generalised estimating equation logistic regression model of final pregnancy status is presented in Table 15. The weighted average predicted proportion pregnant across all herd and parity combinations for cows that calved 3.3 weeks after the planned start of calving (the average) for transiently infected cows was 88.7% and for control cows was 92.7% (p = 0.002). The difference between TI cows and control cows was 4.0%. This would cost approximately 0.04 x \$951 = \$38.04 per transiently infected cow on average.

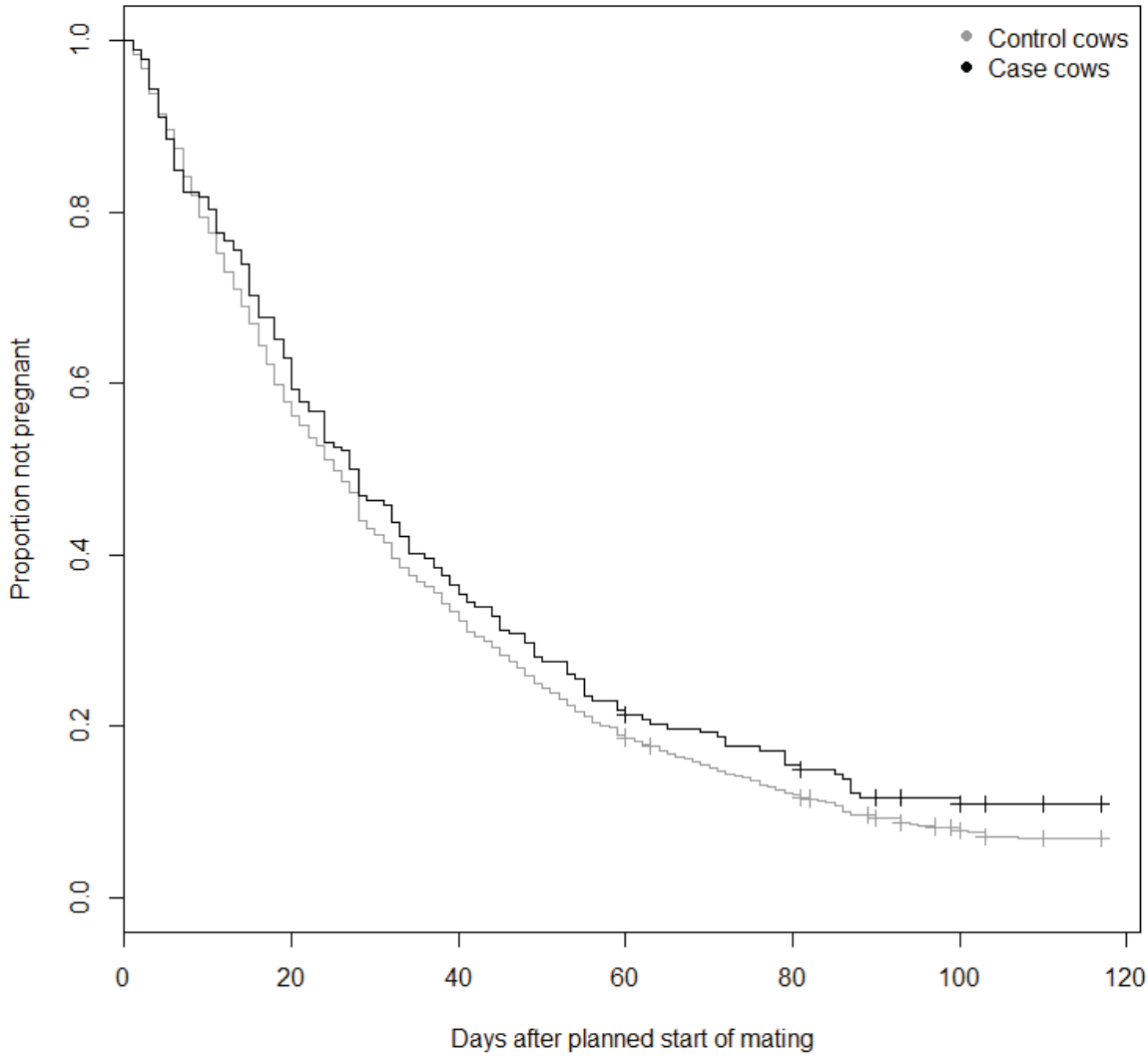

Figure 5: Kaplan-Meier survival curves of the time from the planned start of mating to conception for transiently infected cows (black), and other cows (grey).

Other reproduction models

A summary of the effect of transient infection on the first service conception rate, conception to artificial insemination, 6-week in-calf rate (conception in the first 6 weeks of mating), and abortion, from marginal models, is presented in Table 16. None are statistically significant. First service conception rate, conception to artificial insemination, and 6-week pregnancy all showed a relative risk of 0.93 to 0.95, indicating a 5% to 7% poorer reproductive performance for transiently infected cows than for other cows. Transiently infected cows also had a 49% higher risk of abortion. The cost attributable to reduced conception to AI was 0.06 x $141 = $8.46.

Disease

The hurdle model for the effect of transient infection on the cost of illness is presented in Table 17. The weighted average predicted cost of disease was $13.41 in control cows, and $25.37 in TI cows (1.9 times the cost in control cows). The predicted difference was $11.97. This 1.9 times increase in the cost of disease was over an average of 213 days, so if the effect of transient infection applied for

28 days, the marginal increase in the risk of disease expenditure during that 28-day period would have been approximately 0.9 / (28 / 213) = 6.8 times the base risk. The risk of other disease during the 28 days of transient infection would, therefore, be 7.8 times the risk at other times.

Table 14: AFT model results for the planned start of mating (PSM) to conception interval accounting for cows clustered in herds using a log-logistic distribution.

	OR	Estimate	SE[1]	*p*
Intercept		4.158	0.041	<0.001
TI cows	1.13	0.124	0.087	0.157
calving to PSM	0.98	-0.016	0.001	<0.001
Parity				
> 4	ref	ref	ref	<0.001[2]
1	1.12	0.113	0.077	0.058
2	0.93	-0.068	0.077	0.375
3 or 4	0.83	-0.186	0.053	<0.001

Scale = 0.613, log(scale) $p<0.001$

[1] Standard error.

[2] Likelihood ratio test p-value

Interpretation: After accounting for other factors, transiently infected cows took 13% longer to conceive on average.

Table 15: Final pregnancy status generalised estimating equation model accounting for herd clusters with exchangeable correlation structure

	RR (95%CI)	Estimate	SE	*p*
Intercept		3.023	0.143	<0.001
TI cow	0.95 (0.91-0.99)	-0.481	0.157	0.002
Calving week	0.98 (0.98-0.99)	-0.188	0.011	<0.001
Parity				
5 or more	ref	ref	ref	<0.001[1]
1	0.98 (0.94-1.00)	-0.258	0.148	0.081
2	1.02 (1.00-1.04)	0.279	0.129	0.031
3 or 4	1.04 (1.02-1.06)	0.707	0.236	0.003

[1] Likelihood ratio test p-value

Interpretation: After accounting for other variables in the model, transiently infected (case) cows had a 5% (95% CI 1% to 9%) lower chance of being pregnant at the end of the season that control cows.

Table 16: Summary of the effect of transient infection on reproductive outcomes from logistic generalised estimating equation models accounting for parity and calving to the planned start of mating date[1].

Outcome	n	RR (95% CI)	Estimate	p
First service conception	3,636	0.93 (0.80-1.05)	-0.151	0.232
Conception to AI	3,561	0.94 (0.78-1.08)	-0.187	0.390
Pregnant by week 6	3,561	0.95 (0.83-1.06)	-0.158	0.394
Abortion[1]	2,968	1.49 (0.50-4.38)	0.409	0.474

[1] The abortion model included only cows that had a confirmed conception date and were subsequently pregnancy tested to confirm abortion (or not) during the observation period if culled, and did not account for calving to planned start of mating date.

Table 17: Results of the hurdle model for the risk of disease and, if it occurred, the cost of disease (treatment cost plus discarded milk cost).

Count (cost) component (offset negative binomial):

	IRR[1]	Estimate	SE[2]	p
Intercept		-0.439	0.105	<0.001
TI cow	1.36	0.309	0.151	0.040
Herd				
8	ref	ref	ref	0.002
1	0.96	-0.041	0.155	0.789
2	0.94	-0.061	0.123	0.623
3	2.27	0.821	0.188	<0.001
4	1.54	0.434	0.176	0.014
5	1.09	0.084	0.164	0.608
6	1.11	0.108	0.246	0.659
7	1.38	0.320	0.178	0.071
9	0.84	-0.174	0.145	0.228
10	0.78	-0.247	0.197	0.210

Hurdle component (binomial):

	OR[3]	Estimate	SE[2]	p
Intercept		-2.944	0.168	<0.001
TI cow	1.36	0.305	0.245	0.213
Parity				
5 or more	ref	ref	ref	0.029
1	0.63	-0.455	0.181	0.012
2	0.51	-0.671	0.179	<0.001
3 or 4	0.72	-0.324	0.147	0.028
Herd				
8	ref	ref	ref	<0.001
1	2.83	1.042	0.245	<0.001
2	7.37	1.997	0.196	<0.001
3	1.41	0.342	0.288	0.236
4	2.21	0.793	0.276	0.004
5	2.64	0.972	0.258	<0.001
6	0.98	-0.020	0.378	0.957
7	1.71	0.536	0.275	0.051
9	3.81	1.337	0.231	<0.001
10	1.83	0.606	0.309	0.050

[1] Incidence rate ratio, [2] standard error, [3] odds ratio

Interpretation: Transiently infected cows (TI cows) had 1.36 times the odds of becoming ill after accounting for herd and parity, and of cows that become ill, they had 1.36 times as many dollars spent on them including the cost of treatment and the cost of discarded milk.

Table 18: Summary of the effect of transient infection on various disease measure regression models accounting for parity and herd

	OR[1]/IRR[2]	Estimate	SE[3]	*p*
Mastitis				
Any[4]	1.35[1]	0.302	0.276	0.274
Cases	1.28[2]	0.246	0.289	0.395
Days ill	1.23[2]	0.205	0.524	0.696
Lameness				
Any[4]	1.26[1]	0.230	0.388	0.553
Cases	1.72[2]	0.545	0.349	0.118

[1] Odds ratio from logistic regression model, [2] Incidence rate ratio from a negative binomial regression model with cow time at risk offset, [3] Standard error, [4] binomial outcome: any cases.

Interpretation: Transiently infected cows had 1.35 times the odds of getting mastitis, 1.28 times higher incidence of cases of mastitis, and 1.23 times longer time out of the vat due to mastitis cases compared to other cows with the same parity, after accounting for time at risk for count outcomes, but none of these estimates were statistically significant.

Mastitis was the most frequently recorded disease (334 cows, 61% of cows with some illness), followed by lameness (143 cows, 26% of cows with some illness recorded). None of the single disease models had a statistically significant effect of transient infection. The estimated effect of transient infection on mastitis and lameness are presented in Table 18. Transiently infected cows had 1.35 times the odds of at least one case of mastitis, and 1.26 times the odds of at least one case of lameness compared to other cows from the same herd with the same parity. They also had 1.72 times higher incidence of lameness, 1.28 times higher incidence of cases of mastitis per day at risk, and 1.23 times longer cumulative time out of the vat due to mastitis cases compared to other cows from the same herd with the same parity, after accounting for time at risk. Time out of the vat could not be assessed for lameness because most cases did not result in discarded milk in these herds.

Discussion

This is the first study providing estimates of the proportion of cows seroconverting to BVD virus between the start of the seasonal breeding program and the final production recording (approximate 4 to 5 months) in seasonal calving dairy herds. Seroconversion was associated with a lower final pregnancy rate, probably due to the observed trend of a longer PSM-conception period, and an increased risk of clinical disease. There was a low proportion of susceptible cows at the start of mating (3.8%) in herds with a milking PI, but a large proportion (82.4%) of these cows seroconverted over the observation period of 125d. This seroconversion rate would require 4.6 potentially effective contacts per PI per day. Transiently infected cows cost an average of $11.97 (1.9 times) more than non-TI cows due to treatment costs and discarded milk and had a 4% lower final pregnancy rate. The average cost attributable to transient infection between the start of mating and final herd test was $32.61 for delayed conception + $38.04 for lower final proportion pregnant + $8.46 for reduced conception to AI + $11.97 increased other disease costs = $91.08 per transiently infected cow.

The cost of disease values are a transformed composite of the count of the number of milkings discarded, and the count of the number of treatments with a limited set of treatments (18 different treatments were used for at least one incidence of illness, and 10 treatments were used for at least 5

incidences). These counts were combined by converting them to a dollar-value and the result was a composite count of the cost of the illness with a limited set of integer values, bounded by zero. More expensive, and longer duration of treatment would tend to be chosen by farmers or vets in response to more severe illness, so these values will scale with the severity of illness as well as incidence. This additional information makes this analysis more powerful than standard incidence analyses which showed a similar trend but were not statistically significant (not shown). Because these data are a composite of transformed counts, there was a large excess of zero values, the average cost was close to zero (median $0, mean $12), and negative values are not possible, analysing these data as a continuous outcome was not appropriate. Zero-inflation modelling was considered but since the cost of clinical illness was the outcome of interest, allowing for a zero-cost clinical illness (as implied by that method where zeros can be partitioned into either component) was not appropriate. The hurdle model interpretation of "any illness" for one component, and "degree of illness" for the other was preferred. After accounting for the excess zero values using a hurdle model, the extreme overdispersion relative to a Poisson distribution left the negative binomial as the only valid alternative for the count component (Zeileis *et al.* 2008).

The estimate of the effect of seroconversion in the current study may be an underestimate due to some animals seroconverting in the days or weeks before the study commenced. If BVD virus infections earlier in the season increased prevalence and severity of contagious diseases, it would increase the incidence of disease in other cows and may increase the risk of recurrent disease in the control cows. BVD virus infection occurring ≤9 days before a mating can affect the reproductive performance (McGowan *et al.* 1993a), and it is possible that this effect may persist for longer than 9 days (Ssentongo *et al.* 1980). Any BVD virus attributable effect on disease incidence or reproductive performance in control cows would reduce the apparent size of the effect of BVD.

This study did not differentiate exposures occurring before conception or at various stages of gestation. Therefore, it is difficult to compare the rate of events in experimental studies like McGowan *et al.* (1993a) and Stokstad *et al.* (2003), or conception date aligned field studies (where events are reported relative to gestation stage rather than calendar date) like Rufenacht *et al.* (2001). Our study design accounted for both the size of the effect and the proportion of cows infected at a given stage of gestation in endemic field conditions without the need to model herd and infection dynamics. If exposure followed a typical contact-dependent exponential pattern, a greater proportion of the cows in the TI category would have been exposed early in the mating period, compared to late in the mating period, especially for BTM PCR positive herds without a lactating PI where the period of exposure to the PI may have been limited. Many of the TI cows infected early in the season would not have conceived before infection so would not be eligible for most of the reproductive effects of BVD.

The rates of reproductive loss observed in this study are consistent with effect size estimates of other studies (Houe *et al.* 1993b; Larsson *et al.* 1994; Rufenacht *et al.* 2001) if the variation in the proportion of cows infected in each stage of gestation is considered. Similarly, the effect of transient infection on disease cost is consistent with herd-level studies (Niskanen *et al.* 1995; Fredriksen *et al.* 1998; Valle *et al.* 2001; Robert *et al.* 2004) since the proportion of exposed cows and the stage of lactation in the herd-level studies were unknown. There is a higher risk of disease in the peripartum period which was not included in the observation period of this study so the disease cost estimate in this study will be smaller than it would be in a study that included the peripartum period. The proportion of adverse events and the cost of infection could be much greater in an outbreak situation depending on the stage of the season.

Herds 4-10 had lactating PI cows that remained in the herd throughout the observation period, so the average exposure period of 125 days is appropriate for cows in those herds. However, this value used to estimate the number of contacts per day may be a large overestimate of actual exposure period for herds 1-3 where there was no PI in the lactating herd. Contact with a non-lactating PI would usually be intermittent or of short duration unless the PI was a bull. Given that these herds were BTM PCR positive before the start of mating, and given the much lower seroconversion rates, the infection sources for these herds were probably not PI bulls. If the duration of exposure was overestimated, the probability of contact will be underestimated. It is not possible from this data to distinguish between a shorter duration of exposure, and a lower contact rate, so the duration of exposure that gave the same contact rate as the herds with a lactating PI, and the number of contacts required if there was only a single day of exposure were also reported. Further research on contact rates in lactating dairy cows would help to clarify infection dynamics and inform disease modelling.

Herds were enrolled in the study on the basis of the presence of BVD virus in BTM. However, in three of 10 herds, a lactating PI could not be detected. Seroconversions did occur in these herds, however, which confirms that there was a source of infection on the property or nearby for at least some of the observation period. Persistently infected cattle are recognised as the main source of infection and are usually very efficient at infecting in-contact cattle (Houe 1999), so intermittent contact with a non-milking PI such as a PI calf on the property, or a PI on a neighbouring property are possible explanations. Other possibilities include indirect contact with a PI through fomites (Gunn 1993), airborne transmission from a PI that did not have direct access to the herd (Mars *et al.* 1999; Bitsch *et al.* 2000), and continued virus circulation by transiently infected animals (Moerman *et al.* 1993; Moen *et al.* 2005). However, some studies failed to demonstrate transmission from TI animals (Meyling and Jensen 1988; Niskanen *et al.* 2002), so this may be rare, or strain dependent (Houe 1999). The largest herd (herd 8) had only 1 lactating PI and managed the cows in 3 separate groups (milked through the same shed) but had a very high seroconversion rate indicating that a relatively efficient mechanism of transmission other than direct contact with the PI may have been active in this herd. Although no published data were discovered, the authors wonder if milking equipment may be an important mechanism of transmission within a dairy herd, especially for herds with multiple mobs or where transmission depends on transiently infected cows since the antigen ELISA titres appeared to be comparable to that of PI cows for some of the transiently infected cow milk samples, unlike other potential routes of transmission.

The estimate of a 2.9 day virus shedding period is in line with studies based on serum PCR results (Nickell *et al.* 2011), or nasal and conjunctival virus isolation (Polak and Zmudzinski 2000). This estimate assumed a constant contact rate during the observation period, and that all transiently infected cows were detected. Virus shedding in milk may be less common or of shorter duration than this if the contact rate was higher around the day of sampling (early in the season) than later in the season, so there were more transiently infected cows than expected. If contact rates differed during the season due to sexual activity or other factors, this would be plausible. A shorter duration and lower probability of any shedding would be more in line with shedding rates and levels found with nasal swabs by Niskanen *et al.* (2002) using an immunoperoxidase test, and Nickell *et al.* (2011) using virus isolation. The authors are unaware of any studies investigating transiently infected cows shedding virus in milk, and this may differ from other routes of virus shedding. This information is important for modelling BVD virus infection if allowing for transmission from transiently infected cows.

The high seroconversion rate in herds with a single PI in the milking herd reinforces the point that PI cows are a very efficient source of infection. Only 3.8% of the cows in herds with a milking PI were susceptible at the start of mating, and only 4 to 11 cows seroconverted in all but the largest herd., It is,

therefore, unsurprising that a large proportion of smaller herds don't replace their PIs with the birth of new PI replacements and naturally clear infection as milking PIs are culled or die (chapter 2). The large herd (herd 8) had similar proportions, but the total of 37 seroconversions over the mating period makes it more likely that at least one PI replacement would be created per PI lifetime in the herd. With 1.4% of the herd exposed per day, less than 1% of the herd should remain unexposed after 12 months if the rate remained constant and contacts were random. However, at least some of these herds would have had a PI present in the herd for more than 12 months but still had 1.1% to 5.9% susceptible at the start of mating. Thus, it seems likely that contacts were not simply random, but that social or management factors influenced each cow's probability of contact with the PI. Contact rates may differ at different stages of the lactation cycle (e.g. may be lower when cows are not lactating). Nevertheless, for infection to persist in a herd, there would need to be sufficient susceptible cows entering the herd most seasons to create a new generation of PIs. Replacement heifers entering the herd may fulfil this role in some cases but there was little evidence of parity 1 cows representing a substantial pool of susceptible cows at the start of mating in these herds. Nearly all of the parity 1 cows in these herds were either exposed before entering the milking herd (e.g. during contract rearing on other farms) or had a very high seroconversion rate early in the season. It is also possible that immunity wanes over time in the absence of contact with a PI so that some cows become susceptible again over time, but if this occurs, it is likely to take more than 3 years with no contact (Fredriksen *et al.* 1999).

There is a risk of misclassification which is probably more acute with the smaller ELISA S/P values and change thresholds required for milk samples (Weir *et al.* 2013). Increasing the change in S/P ratio defined as seroconversion would increase the specificity, but decrease the sensitivity for detecting seroconversion. This would reduce the apparent incidence, and thus reduce the power of the study. Other potential sources of misclassification include sample contamination, test variation, and human error reading or reporting tag numbers. Misclassification errors would dilute the effects of transient infection if naïve or previously immune cows were classified as transiently infected, or reduce the power and increase the risk of type 2 errors if transiently infected cows were classified as immune or naïve. These misclassification errors may have contributed to the failure of some of the models to demonstrate a statistically significant effect of transient infection but are unlikely to have caused bias in the means of TI-specific cost estimates. The authors recommend using serum samples where possible for future studies of this nature despite the increased invasiveness and challenging, time-sensitive logistical issues for large herds. In addition to the risk of misclassification affecting the power, there were a relatively low number of transiently infected animals in this study despite the large number enrolled because of the small number of initially susceptible animals in herds with a lactating PI. The existence of an effect of transient infection on reproductive performance is well established (McGowan *et al.* 1993a; Grooms 2006), so non-significant results were still reported. Also, since the effect of transient infection on other disease cost was significant, the effect on mastitis rate, and possibly lameness, are also likely to be real.

Chapter 6: General discussion

Before undertaking this research, there was little understanding of, or agreement about, BVD control in New Zealand. A voluntary industry committee (The BVD steering committee) had concluded that "a formal control programme was not feasible at [that] time due to limitations in diagnostic techniques, industry awareness, and economic impact data", and there was division within the veterinary profession about what could or should be done[1]. There was some New Zealand prevalence data (Thobokwe *et al.* 2004; Compton and McDougall 2005), a non-peer reviewed study of the effects of persistent infection in New Zealand (Voges *et al.* 2006), and a herd-level study estimating the economic cost of fertility, production, and PI losses (Heuer *et al.* 2007). Most of the effects of BVD virus infection on naïve individuals were well established from overseas research (Baker 1995; Lindberg 2003; Grooms 2006), with the possible exception of the impact of immunosuppression in lactating cows. However, no data were available on the effect sizes in New Zealand. Additionally, there was no information about infection dynamics within and between seasonal calving, pasture-based dairy herds. Quantification of effect sizes and infection dynamics in New Zealand was required to plan control strategies and optimise the use of limited resources, as well as being prerequisites for the development of a comprehensive BVD model to assess the cost-benefit of control in New Zealand dairy systems. This thesis therefore aimed to quantify the herd infection dynamics, compare bulk tank PCR and antibody ELISA, quantify the relative importance of risk factors associated with herd infection status, optimise cut off values for individual milk ELISA testing, assess cow-level infection dynamics, and provide local estimates of the effects of transient infection in lactating dairy cows.

Since 2007 when these studies commenced, the BVD situation in New Zealand has changed substantially. In 2011, the BVD steering committee released the BVD Management Toolkit[2] and held regional meetings for veterinarians throughout New Zealand[3] to introduce the toolkit and encourage a consistent approach to BVD control. This toolkit and the meetings, which more than one-third of registered large-animal veterinarians attended, along with another round of meetings in 2014 which were also well attended, has contributed to a greater level of awareness, confidence and consistent approaches to voluntary BVD control in New Zealand. The author contributed to the development and presentation of the Toolkit and drew extensively on the results of this research.

The bulk tank milk (BTM) PCR used for the BVD herd study in 2007 (Chapters 2 and 3) was the first time that a BTM PCR was used on commercial farms in New Zealand. The current studies results have improved the interpretation of BTM BVD tests. Since then, the use of bulk tank BVD testing has rapidly increased with approximately 70% of New Zealand dairy herds undertaking bulk tank BVD testing in 2015 (Hinrich Voges 2015, personal communication). This is an indication of the increasing level of interest and understanding of BVD control by New Zealand dairy farmers, although the proportion of herds with a systematic control program is considerably smaller than the proportion testing. With the availability of a BTM PCR, and pooled serum PCR to reduce the cost of individual screening for PIs, the barrier of the perceived "limitations of diagnostic techniques" has largely been removed. The insights reported in this thesis have been widely extended to dairy veterinarians through conferences and regional extension meetings, and contributed to improved confidence and quality of advice to herd managers.

The highly dynamic BVD virus infection situation in smaller herds, where infection is naturally cleared between seasons more frequently than in large herds, has important implications. These small

[1] http://www.controlbvd.org.nz/
[2] http://www.controlbvd.org.nz/bvd-management-toolkit
[3] http://www.controlbvd.org.nz/technical-information

herds are less likely to benefit from active clearance of infection, particularly late in the season since 67% of them cleared infection from the milking herd spontaneously without active intervention. Conversely, larger herds are unlikely to clear the infection between seasons so active intervention is important for them. The relatively large proportion of herds experiencing BVD virus incursions each year indicates that actions to prevent introductions or quickly detect and remove them will be more valuable than if infection status was more stable. The data generated from the current study concerning the variation in infection dynamics amongst herds will be useful for tuning and validation of a simulation model. Since the economic impact of a BVD outbreak is likely to be much greater in naïve herds, and a number of these herds experienced introductions (chapter 2), they will benefit from controlling new BVD virus introduction through the biosecurity measures described in the Toolkit. Understanding the expected change of antibody level between seasons allows veterinarians and herd managers to interpret serial BTM testing results and recognise when there is evidence of virus exposure due to ineffective biosecurity.

The distribution of many of the questionnaire variables related to herd dynamics and management systems described in the descriptive results section of chapter 3 would be required inputs for simulation models. These descriptive results describe the pattern of management practices on New Zealand farms which may also be relevant for other applications. Understanding the relative importance of the risk factors for BVD virus herd infection status is an important prerequisite for designing cost-effective control measures. This study demonstrated associations between BVD virus infection status and all of the expected risk factors for BVD virus infection except for the risk from purchased breeding bulls and the risk from indirect contact through fomites. The risk from bulls was considered an important risk *a priori*, but was not significant in our data, possibly due to extensive control of this risk already in place, recall bias, and misclassification. Some of the effect of indirect routes of virus introduction into a herd may be attributed to other factors included in the models like location and animal movements. The risk from indirect spread includes potential sources of infection that may be classified as 'minor risk' (various fomite-mediated routes). It may be difficult to demonstrate this in an observational study. Nevertheless, it is generally accepted in New Zealand that indirect spread contributes to virus introduction but this is difficult to prevent completely. However, vaccination is a means for mitigating the impact of virus introduction to a naïve herd, and calf screening is likely to detect potential breakdowns of biosecurity before a resulting PI calf exposes the rest of the adult herd (beyond the small number initially exposed to create the PI calf).

The important infection routes in decreasing order of importance were purchased cows and their calves, contact with neighbour's stock over the boundary fence, and stock movements on and off the home farm (agistment). The initial relevance of these results was to strengthen advice that purchased cows and their calves should be tested since they were the most important source of infection. It also highlighted the fact that neighbour's stock are an important risk that should be mitigated. The relative importance of these transmission routes is relevant for designing and validating any BVD disease model that allows for the introduction of infection into a herd. The other factors that were associated with BVD virus infection were large herd size, lack of herd BVD vaccination, not testing purchased cows, and share milking (compared to owner managed). These factors had plausible relationships with infection status and enhanced the understanding of infection dynamics and risks in New Zealand dairy herds, and provide further insights and potential points of validation for a BVD model.

The diagnostic threshold of the IDEXX serum antibody ELISA appears to have been calibrated for serum but not milk samples. This thesis (chapter 4) demonstrated that the serum cut off values were not valid for milk samples. Hence, the ELISA S/P cut-off values were modified by comparing serum and milk antibody levels. The adjusted cut-off values proved to be essential for defining serological

status accurately (chapter 5). There were no statistically significant effects when transient infection was defined by the original cut-off values due to misclassification (not shown). With the availability of bulk tank antibody ELISA and PCR, and pooled PCR screening for groups of individuals, New Zealand BVD control schemes are unlikely to rely on individual antibody testing as was common in the BVD control schemes of the 1990's (Lindberg and Alenius 1999). There are situations where individual screening may be useful, however, and milk may be a relatively cheap and convenient sample type in some of these situations. For example, if testing cows where the owner wants to vaccinate only susceptible cows, to inform the decision whether to vaccinate or not based on the proportion susceptible, during an investigation into the source of infection where cows have been combined from multiple locations, or for research. Having more appropriate cut-off values facilitates milk testing, but the author would recommend against it if serum sampling is a viable alternative. This knowledge may be valuable for designing future research.

The estimation of the total cost of BVD virus infection from cow-level data would need to account for the proportion susceptible and the rate of seroconversion within a herd. Modelling infection dynamics would require an estimate of the number of effective contacts per day. These rates and proportions were estimated in the transient infection study (chapter 5). The proportion immune at the start of the mating period and the proportion of susceptible cows that seroconverted during the mating period in herds with a lactating PI were quantified for the first time and were both larger than expected (Thompson 2005). These proportions contradict some assumptions commonly expressed by advisors in conversations with the author, such as the expected proportion of reproductive losses, and the probability of finding PI replacement calves in herds where BVD virus is endemic. There is a limited amount of information about transiently infected cow shedding in nasal and conjunctival excretions (Polak and Zmudzinski 2000; Niskanen *et al.* 2002; Nickell *et al.* 2011), but the author is not aware of any prior information about shedding in milk. The infectious period of TI cows is another required parameter for disease modelling if transmission from transiently infected cows is included. The reproductive effect size estimates for transiently infected cows are novel because they account for endemic infection dynamics in seasonal calving herds, but the existence of the reproductive effects was previously established (McGowan *et al.* 1993a; Moennig and Liess 1995). The increase in illness cost in transiently infected lactating cows was substantial and provides new information. Converting the observed increased risk of illness (RR) to apply to a more plausible immune suppression period instead of the total observation period was also a novel approach and allows the application of this effect to other situations such as a different time at risk, or the inclusion of immune suppression effects in a BVD model. The summary cost of transient infection in lactating cows in endemically infected seasonal calving herds was interesting but underestimates the total cost as it represents only part of the cost over part of the year in endemically infected herds. Measuring the cost in endemically infected herds throughout the year would require a study following herds over 2 seasons because it is not realistic to identify numerous infected herds before lactation begins. In addition, more than twice as many herds would be required since many clear infection between seasons and on-going seroconversions would lead to very few susceptible cows. A more cost-effective approach would be to use the information provided here for disease modelling. The cost in outbreak scenarios and the cost-benefit of control will also require comprehensive modelling which will take advantage of the insights provided by this research.

This thesis has provided answers for the most important epidemiological questions about BVD in New Zealand, yet some knowledge gaps remain. Aspects of BVD in New Zealand for which further investigation is desirable include: the calf growth effects associated with proximity to a PI (Hessman *et al.* 2009); the milk production effect (Heuer *et al.* 2007) of transient infection, or possibly exposure

of immune cows to a PI (similar to the calf growth effect); the impact of late-gestation infection on subsequent growth rates and reproductive performance (Munoz-Zanzi *et al.* 2003; Munoz-Zanzi *et al.* 2004); the capacity for milk to transmit infection between lactating dairy cows; and contact rates within groups of cows and cross-boundary with neighbour's stock (important for modelling BVD). Observational studies of most of these aspects would be impractical or cost-prohibitive due to the difficulty identifying suitable groups containing PI animals and the difficulty managing confounding factors between different groups on pasture. Experimental approaches where PI animals are introduced to naïve groups with carefully controlled conditions may be feasible. The most important remaining knowledge gap is an economic cost-benefit analysis of BVD control. Therefore, a comprehensive BVD cost-benefit model is an ideal focus for future research. This would be an important tool for industry decision-making.

Conclusions

New Zealand has seen many changes and improvements in BVD control since the 2005 BVD symposium and this research has contributed to those improvements. The initial benefit for the New Zealand dairy industry came from the application of these results to voluntary BVD control strategies. The most important gap in our knowledge that prevented industry engagement with extension and coordinated control efforts was the economic case for control and the cost-benefit of BVD control approaches. This gap can be filled by use of a comprehensive BVD model, and most of the missing information required to build such a model was generated in this thesis. The differences in infection dynamics between large South Island herds and smaller North Island herds are relevant to BVD control decision-making and will be useful for tuning and validation of a BVD model. The relative importance of transmission pathways is also useful for control decision-making and will be important for tuning and validation of a simulation model. There may be some commercial applications of the individual antibody ELISA cut-off value adjustment, but the most important application is for research, particularly the transient infection research in this thesis. The proportions of initially susceptible and transiently infected cows in herds with and without a lactating PI has important implications for endemically infected herds and will be very important, along with the PI contact rates, for a BVD model. The estimate of the increase in other disease cost was novel, and the cost of transient infection during the mating period in endemically infected herds is relevant for control investment decisions but represents only part of the cost in endemically infected herds. To estimate the average annual cost or the total cost of BVD at a national level, and the benefit of various control strategies, a comprehensive cost-benefit model incorporating these findings is required.

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Appendix 1: Questionnaire

Contact Details

Name: ..... **Date:** ...../...../2009

Email: ..... **Processor and Supply number:** .....

General Stock Details

1. When do heifers and cows **calve** in your herd? ☐ Spring only ☐ autumn only ☐ spring and autumn

☐ other (including year-round and extended lactation, please specify) .....

2. What was/is the **number of all age cows** to calve in spring 2008....., and 2009.....

3. How many of these were/are **heifers** entering the herd in 2008 ....., and 2009 .....

4. What was the **number of empty** cows in autumn 2009 (including those culled before pregnancy testing that were suspected of being empty)? .....

5. What was the **number of empty heifers** in autumn 2009? .....

6. How many cows and heifers had calved by 42 days after planned start of calving 2008? .....

7. If the milking herd is **usually split**, what **mob sizes** are they split into? .....
If split, on what basis are they split (eg. Calving date, age…)? .....

8. What is the **effective land area** of your farm (include units)? .....

9. What are the **dominant** breeds (more than 40% of your herd)? **(Please tick)**

Holstein/Friesian ☐
FxJ Crossbred ☐
Jersey ☐
Ayrshire ☐
Other (please specify) .....

10. What was the **mating start date** in 2008? ...../...../.....

11. What date did **AB finish** in 2008? ...../...../.....

12. What was the **last possible mating date** (when the bulls were taken out)? ...../..... /.....

13. How many **abortions** were there last year *(either confirmed pregnant by PD and did not calve or were seen aborting)*? .....

14. Have you had a **case of BVD diagnosed** (by lab test) in any of your stock before? Yes ☐ No ☐

If **Yes**, roughly when was the last case diagnosed? ...../...../.....

15. How many full time equivalent labour units are there working on your farm (including yourself, partial units OK)? .....

continued over page…

16. Please indicate your position of management:

Owner/operator ☐
Farm Owner with a manager ☐
Farm Owner with a share milker ☐ → percentage: .....
Share milker ☐ → percentage: .....
Manager ☐

Stock Movements

17. For each class of stock, please use the table below to indicate the status of **stock movement off your farm** and back **(Please tick all appropriate boxes)**

	Don't leave the farm	Away and completely isolated	Away but may have contact with other stock over a fence	Mixed with stock from other farms	Stock go away to shows
Calves	☐	☐	☐	☐	☐
Heifers	☐	☐	☐	☐	☐
Cows	☐	☐	☐	☐	☐
Carry-overs	☐	☐	☐	☐	☐

18. Are calves kept from any heifers (as replacements or for beef etc.)? Yes ☐ No ☐

19. Are calves (including for beef) kept from any cows that go off the farm during the first 4 months of pregnancy (including carry overs)? Yes ☐ No ☐

20. Were calves (including beef calves) kept from any carry-over cows? Yes ☐ No ☐

21. If calves **did go out grazing**, when did they leave the farm? ...../...../.....

22. Were calves ever grazed together with cows in a **hospital paddock**? Yes ☐ No ☐

23. Which term best describes your calf management after weaning: **(Please tick)**

A few to a paddock in contact with milking herd ☐
Mob(s) rotationally grazed in front or behind the milking cows ☐
Calves always grazed separately from cows (no joint grazing of calves and cows) ☐
Other (please explain)

.....
.....

Bulls

24. How many bulls came on to this farm for the first time in the last 12 months? .....

25. How many were: calves ..... yearlings ..... 2-year-olds .....
3-year-olds ..... 4-year-olds ..... older .....

26. Were the bulls:

Born on the farm ☐
Bought, leased or borrowed ☐

Continued next page

27. How long did/will the bulls stay on the farm?

Mating period only ☐
2 or 3 years ☐
4 or more years ☐

28. Were all bulls tested for **BVD** virus ("antigen" or "PCR")? **(Please tick)**

Before arriving ☐
After arriving ☐
Not tested ☐
Unknown ☐

29. How many **bulls** were in with the **milking herd** at any one time for mating? .....

30. Were the bulls swapped in & out regularly (ie. every 1-3 days)? Yes ☐ No ☐

Cows

31. How many cows were brought onto this farm from another farm (eg. bought, borrowed, leased) for the 2008 season ....., and for the 2009 season ..... (**If none, go to question 34**)

32. Were these cows **tested** for BVD virus? **(Please tick)**

Tested all ☐
Tested some ☐
Tested none ☐

33. Were **calves kept** from any of these cows? Yes ☐ No ☐

34. How many carry-over cows did you retain from last season? .....

35. Would this be roughly ☐ more ☐ less ☐ or the same as previous years?

36. When new stock arrive or your stock arrive back onto the farm after grazing off, are they kept separate (quarantined) for 3 weeks or more from any cows that remained at home? ☐ Yes ☐ No

Please continue over page…

37. How many **paddocks** are there on your farm? .....

38. How many paddocks could allow **over the fence contact with neighbours stock**? .....

39. How many neighbouring properties allow nose to nose contact over the fence? .....

40. If and when contact occurs, how many cattle actually show interest in the contact? **(Please tick)**

Don't know ☐
None ☐
Few (1 to 5) ☐
Some (6 to 20) ☐
Many (more than 20) ☐

41. Please tick all stock classes that were vaccinated for BVD in the last 12 months:

Calves ☐, Heifers ☐, Cows ☐, Bulls ☐, New-comers ☐, **or** None ☐

42. Having shared your time and information, is there anything that has not been asked that you think might be relevant?

……

……

Thank you for taking the time to fill out this questionnaire.
Please:

- **Check that all questions have been answered (put '0' or a line through any that don't apply so we know that you haven't just missed it)**
- **Sign the authorization for bulk tank milk testing (below)**
- **Post all pages (except the letter) back in the pre-paid envelope at your earliest convenience.**

10000

Authority to Test Bulk Tank Milk

This form is to give permission for your processor and their contracted laboratory to pass bulk tank milk samples from your herd for testing (at no cost to you). It will be tested for BVD virus and BVD antibodies. This sample collection will be done roughly 2 weeks prior to your start of mating and will be repeated next year around the same time. The results will be passed on to Eltham Vet Services and will be used for research purposes. You and your vet will receive the results along with relevant information after the second test.

"I have the authority to give permission for this herd to be tested and I hereby authorize my processor and any agents thereof to pass bulk tank milk samples on to a third party laboratory for the purposes of BVD testing, and the laboratory to send the results to Eltham Vet Service. These results and the questionnaire (attached) may be used for research and reported as long as I am not specifically identified."

Signed: ..... Date: .....

Name: ..... Supply number: .....

Appendix 2: Statement of contribution for chapter 2

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Andrew Weir

Name/Title of Principal Supervisor: Cord Heuer

Name of Published Research Output and full reference:

Not published

In which Chapter is the Published Work: 2

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Recruited and enrolled 75% of the herds, conducted all analyses, primary author responsible for writing the article and integrating suggested edits from co-authors.

Andrew Weir Digitally signed by Andrew Weir Date: 2017.02.15 16:17:28 +13'00'

Candidate's Signature

15/2/2017

Date

Cord Heuer Digitally signed by Cord Heuer DN: cn=Cord Heuer, o=Massey University, ou=EpiCentre, email=c.heuer@massey.ac.nz, c=NZ Date: 2017.02.15 14:13:20 +13'00'

Principal Supervisor's signature

15/2/2017

Date

Appendix 3: Statement of contribution for chapter 3

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

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Name of Candidate: Andrew Weir

Name/Title of Principal Supervisor: Cord Heuer

Name of Published Research Output and full reference:

Not published

In which Chapter is the Published Work: 3

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Recruited and enrolled 75% of the herds and collected and validated their questionnaires, conducted all analyses, primary author responsible for writing the article and integrating suggested edits from co-authors.

Andrew Weir Digitally signed by Andrew Weir Date: 2017.02.15 16:18:22 +13'00'

Candidate's Signature

15/2/2017

Date

Cord Heuer Digitally signed by Cord Heuer DN: cn=Cord Heuer, o=Massey University, ou=EpiCentre, email=c.heuer@massey.ac.nz, c=NZ Date: 2017.02.15 14:14:07 +13'00'

Principal Supervisor's signature

15/2/2017

Date

Appendix 4: Statement of contribution for chapter 4

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Andrew Weir

Name/Title of Principal Supervisor: Cord Heuer

Name of Published Research Output and full reference:

Weir A, Heuer C, McDougall S, Voges H. Use of an enzyme-linked immunosorbent assay for detecting bovine viral diarrhoea virus antibodies in individual cow milk samples. New Zealand Veterinary Journal 61, 305-9, doi:10.1080/00480169.2012.757729, 2013

In which Chapter is the Published Work: 4

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Collected approximately half of the samples, conducted all analyses, primary author responsible for writing the article and integrating suggested edits from co-authors.

Andrew Weir Digitally signed by Andrew Weir Date: 2016.04.29 08:51:35 +12'00'
Candidate's Signature

29/4/2016
Date

Cord Heuer Digitally signed by Cord Heuer DN: cn=Cord Heuer, o=Massey University, ou=EpiCentre - IVABS - CoS, email=c.heuer@massey.ac.nz, c=NZ Date: 2016.04.29 12:37:00 +12'00'
Principal Supervisor's signature

29/4/2016
Date

GRS Version 3– 16 September 2011

Appendix 5: Statement of contribution for chapter 5

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Andrew Weir

Name/Title of Principal Supervisor: Cord Heuer

Name of Published Research Output and full reference:

Not published

In which Chapter is the Published Work: 5

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Recruited and enrolled the herds, gathered and validated the data, conducted all analyses, primary author responsible for writing the article and integrating suggested edits from co-authors.

Andrew Weir Digitally signed by Andrew Weir Date: 2017.02.15 16:19:32 +13'00'

Candidate's Signature

15/2/2017

Date

Cord Heuer Digitally signed by Cord Heuer DN: cn=Cord Heuer, o=Massey University, ou=EpiCentre, email=c.heuer@massey.ac.nz, c=NZ Date: 2017.02.15 14:14:48 +13'00'

Principal Supervisor's signature

15/2/2017

Date