

**EPIDEMIOLOGY OF BOVINE VIRAL DIARRHOEA VIRUS
INFECTION IN NEW ZEALAND DAIRY HERDS**

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ABSTRACT

This dissertation summarises the findings of two separate studies relating to the epidemiology of bovine viral diarrhoea virus in New Zealand dairy herds.

The objective of the first study was to estimate the distribution and causes of abortion in dairy herds across New Zealand during the 2001/2002 breeding season.

A questionnaire survey was sent to all veterinary practices that were listed as members of the New Zealand Veterinary Association. Veterinarians were requested to indicate the numbers of dairy herds where the rate of aborting cows was None (0%), Low (1-5%) and High (>5%).

The main causes of abortion among a total of 52 herd submissions were *Neospora caninum* (36.5%) and bovine viral diarrhoea virus (BVDV) (17.3%). This frequency distribution was similar in 35 herds submissions from the Low (1 to 5%) abortion category in that 34% were due to *Neospora caninum* and 15% were due to BVDV.

We concluded that BVDV and *Neospora caninum* are the most frequently diagnosed causes of abortion in dairy herds in New Zealand with a problem of abortion, as well as in herds with a low level of abortion.

The aim of the second study was to assess the relationship between results of a bulk tank milk antibody ELISA for bovine viral diarrhoea virus (BVDV) and the prevalence of BVDV sero-positive young stock as an indication of active infection of the herd.

Bulk tank milk samples from 724 dairy herds in the Waikato, Bay of Plenty and Northland regions of New Zealand were tested for BVDV antibodies. From this random population subset, 20 herds were again randomly selected from each of the quartiles of the percentage sero-positive (%SP) ELISA result and contacted for blood sampling. ELISA antibody test results on blood of 15 calves aged 6 – 17 months from each of 50 herds were available for final analysis.

Based on the blood results, 34 herds were classified as non-infected (0-3 sero-positive among 15 calves) and 16 herds as infected (5-15 sero-positive among 15 calves). Receiver operator characteristic (ROC) analysis suggested an optimal cut-off for bulk tank milk of 80 %SP yielding 81.2% sensitivity and 91.2% specificity.

We concluded that bulk tank milk could be used to determine the BVDV infection status of dairy herds in New Zealand.

The results of these studies are presented as two separate enclosed papers.

ACKNOWLEDGEMENTS

A friend of mine once told me that ambition takes a man long distance. Well this one took me to where the world starts, or rather, where the day literally starts. Here I met a group of professional people who are up early in the morning, everyday, before everyone else in the whole world. Although they did not teach me to wake up early in the morning (due to jet lag excuses), they instilled in me a range of skills from English writing, computer skills to basic life skills. Above all they broadened my scope of understanding of statistics, science and veterinary science. To Professor Roger Morris, Professor Peter Davies, Dr Ron Jackson, Dr Cord Heuer, Nigel Perkins, Mark Stevenson, David Lawton, Simon Verschaffelt, Julie Dunlop, Colleen Blair, Diane Richardson, Daniel Russell, thank you, thank you and thank you.

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Most of all I would like to express my thanks and my love to the most important people in my life, my family. My wife Phumzile, my daughters Fisiwe and Lorato, thank you very much for your patience, moral support and unconditional love.

DEDICATION

Konkome
Lorato

Always wished you were here with me in your different respective ways.

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PAPER I.

G Thobokwe and C Heuer. Infrequent abortions are also associated with bovine viral diarrhoea virus (BVDV) and *Neospora caninum*, *New Zealand Veterinary Journal*, Accepted (October 2002)

PAPER II.

G Thobokwe, C Heuer and D Hayes. Validation of a bulk tank milk antibody ELISA test to detect active infection with bovine viral diarrhoea virus (BVDV) in New Zealand Dairy Herds, *New Zealand Veterinary Journal*, Submitted

LITERATURE REVIEW

LITERATURE REVIEW

INTRODUCTION

Since the initial description of acute enteric disease caused by bovine pestivirus by Olafson et al. (1946), bovine virus diarrhoea virus has become an important pathogen in cattle. The virus is associated with a wide range of clinical signs, and epidemiological studies associate the virus with serious economic production losses (Houe 1999).

THE AGENT – BOVINE VIRUS DIARRHOEA VIRUS

Bovine virus diarrhoea virus (BVDV) is a member of the Pestivirus genus within the family Flaviviridae. The other members of the genus that are closely antigenically related are Border Disease Virus (BDV) and Classical Swine Fever Virus (CSFV) (Murphy 1999), see Figure 1 below. Two species of BVDV have been genetically classified as BVDV-1 and BVDV-2 (Valle 2000). BVDV-1 viruses have at least ten serologically distinct subspecies, 1a-1j, and have a worldwide distribution (Table 1). BVDV-2 viruses are as yet largely restricted to the USA and Canada (Vilcek 2001).

BVDV Classification

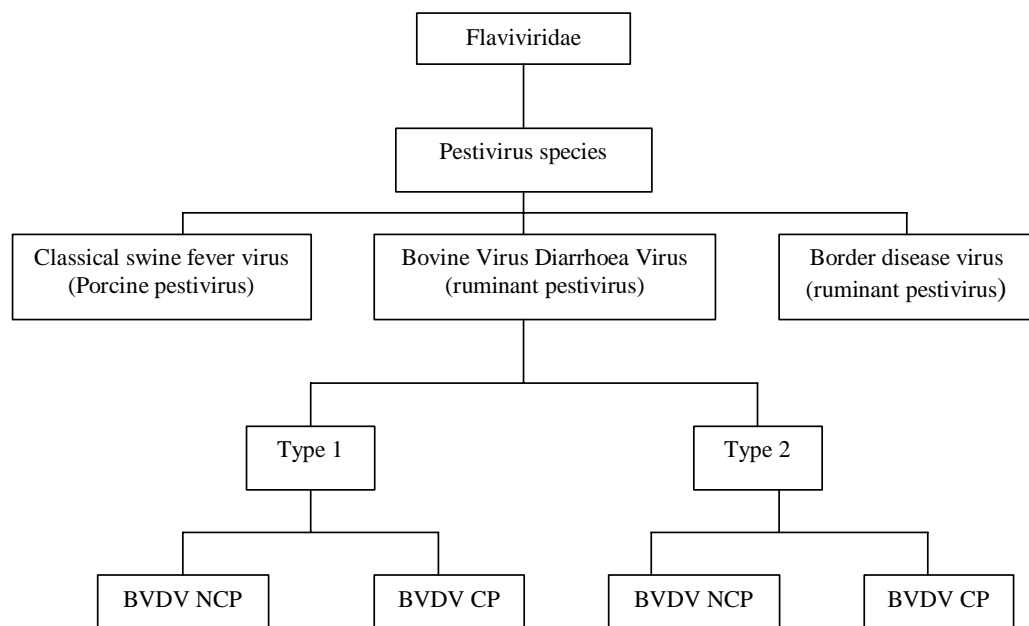


Figure 1. Showing BVDV classification. Brownlie (2000)

Table 1. Distribution of BVDV-1 cattle isolates and strains originating from 13 countries into 10 genetic groups. Vilcek (2001).

Country of origin	No. of viruses	Number of viruses in each generic group									
		a	b	c	d	e	f	g	h	i	J
Austria	23	-	7	-	2	-	10	3	1	-	-
Canada	10	5	5	-	-	-	-	-	-	-	-
France	23	3	6	-	1	13	-	-	-	-	-
Germany	7	1	1	-	2	-	-	-	1	-	-
Hungary	5	-	2	-	-	-	3	-	-	-	-
Italy	6	-	4	-	-	1	1	-	-	-	-
New Zealand	6	6	-	-	-	-	-	-	-	-	-
Mozambique	23	8	2	-	-	-	6	1	3	-	3
Slovakia	3	-	-	-	1	-	1	-	1	-	-
Spain	8	1	7	-	-	-	-	-	-	-	-
Sweden	17	1	6	-	10	-	-	-	-	-	-
United Kingdom	20	17	1	-	-	-	-	-	-	2	-
USA	17	7	10	-	-	-	-	-	-	-	-

BVDV virion, although mainly spherical is pleomorphic in nature, has a diameter of about 45-50nm and has a tightly adherent envelope. The genome is made up of a single molecule of linear positive-sense single-stranded ribonucleic acid (RNA) molecule. The size of the pestivirus genomic RNA was estimated by gel electrophoresis to be 12-13 kilo bases (kb) in size (Collett 1992). The genome encodes a single polyprotein that is proteolytically processed during virus replication to produce a variety of viral structural and non-structural proteins (Murphy 1999).

Of the structural proteins, the major envelope glycoprotein E2 is the most immunodominant and most variable (Toth et al. 1999). It is the major target for neutralising antibodies, which confer protection following infection or vaccination. The highly variable nature of epitope-rich regions within the E2 sequence, dictate that the E2 protein is the most important source of antigenic variability between different BVDV strains (Figure 2).

The most studied non-structural protein is the NS3 protein associated with the lytic activity of the cytopathic strain. This protein forms the basis of several commercially available antibody and virus detection assays.

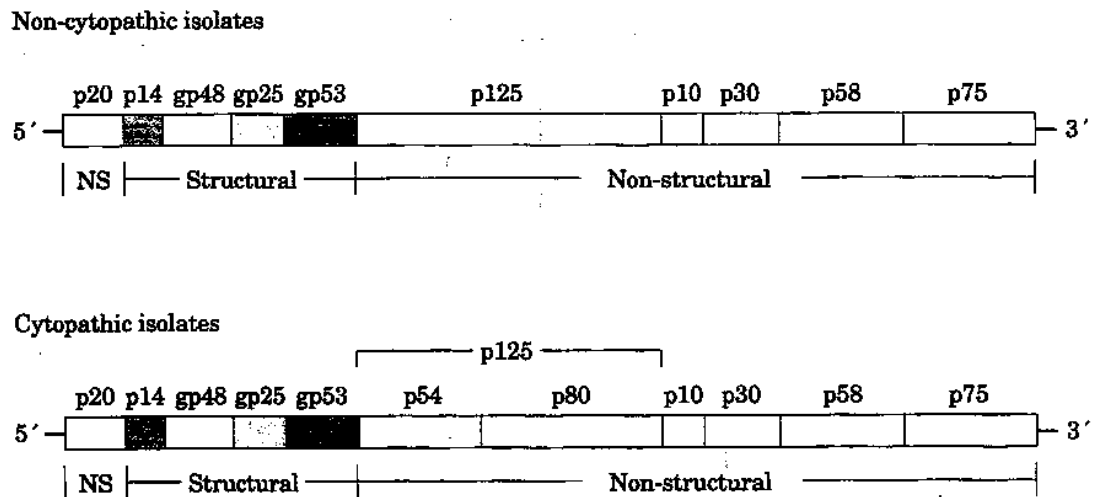


Figure 2. Diagrammatic representation of final protein products of non-cytopathic (NCP) and cytopathic (CP) BVDV. Anderson (2000)

BVDV exist as two distinct biotypes, cytopathic (CP) or non-cytopathic (NCP) (Figure 1). NCP biotype causes no visible cell cytopathology in cell-culture systems. CP biotype causes cellular vacuolation and cell death in cell culture system e.g. bovine testicle, kidney, foetal lung or nasal turbinate cells. Three tissue culture passages are considered necessary to designate an isolate as either CP or NCP (Deregt and Loewen 1995). This laboratory classification is not indicative of the pathogenicity of the biotypes. The NCP biotype persists and circulates in cattle populations. It is usually involved in various manifestations of the disease where as the CP biotype is involved in causing mucosal disease (MD) through superinfection of the persistently infected calf. The two biotypes have been shown to have different tissue tropism (Clark et al. 1985). CP biotype has been readily isolated from the rumen, reticulum, small intestine, mesenteric lymph node, Peyer's patch and colon. NCP has been more commonly isolated from blood and blood rich organs such as nasal cavity, lung, liver, kidney and spleen. Serological studies have shown that final protein and functions of both biotypes are different (Brownlie 2000).

The virus is not stable in the environment. The virus is easily inactivated by heat and can only survive for one hour at 56 °C. At room temperature it survives for up to 5 days

but the virus can be stored for up to 16 months at -40°C . The virus survives best at pH range 5.7 to 9.3 with maximum viability at pH 7.4. Common disinfectants like phenols and chlorhexidine readily inactivate the virus (Murphy et al. 1999).

PATHOGENESIS AND CLINICAL SIGNS

Depending on the immunological competence of the host, the biotype and virulence of the virus, the physiological status of the animal, and the environment the animal exist in, the sequelae to BVDV infection can be divided into three main categories:

- Infection in susceptible immunocompetent non-pregnant cattle - BVD
- Infection in pregnant cows
- Infection in persistently infected cattle- Mucosal Disease (MD)

Infection in susceptible immunocompetent non pregnant cattle- BVD

BVDV infection in non-pregnant cattle is generally mild and is often unnoticed by the farmer. It occurs very commonly with an estimated 60% of cattle in New Zealand reported to be seroconverting. In the United Kingdom (UK) about 95% of milking herds are reported to have seroconverted to BVDV (Brownlie 2000). With acute infection there is inevitably pyrexia, a leukopenia from about days 3 to 7 post infection and a mild nasal discharge. There is usually very limited recovery of the virus from the blood and nasal secretions lasting about 3 to 14 days post infection. This is followed by a rise in antibodies, which reach a maximum 10 to 12 weeks post infection and immunity is assumed to last a lengthy period, possibly the animals life time (Duffell and Harkness 1985; Fredriksen et al.. 1999). It is clear that BVDV can under certain circumstances cause severe clinical disease. The original description of the disease was profuse watery diarrhoea in adult cattle (Olafson et al.. 1946). Episodes of agalactia and diarrhoea have also been reported. Severe and fatal disease has also been reported in the United Kingdom and America with morbidity and mortality reported to be 40% and 20% respectively (Brownlie 2000; Alves et al. 1996).

The virus first replicates in the nasal mucosa, then disseminates to the tonsils and then spreads to the regional lymph nodes followed by a general viraemia (Bruschke et al., 1998). The initial replication in the nasal mucosa may account for the mild oculonasal discharge and stomatitis and erosions observed in some acute infections.

Infection in pregnant cows

BVDV rarely infects the foetuses of seropositive cows. Foetal infection has only been shown as a result of viraemia or acute persistent infection in seronegative animals. This is however the most epidemiologically significant BVDV infection (Valle 2000; Houe 1999; Radostits and Littlejohns 1988). The NCP BVDV invades the placentome and replicates in the trophoblast before crossing to the foetus. The outcome of foetal infection however depends on the stage of pregnancy (Tremblay 1996)

Early embryonic deaths, infertility and repeat breeder cows are frequent sequelae to pestivirus infection during pregnancy. In one study of a herd infected with BVDV, conception rates were reduced from 78.6 percent in the immune cows to 22.2 percent in infected cattle (Virakul et al., 1988). In another study, BVDV at the time of conception reduced pregnancy rates at 77 days from 79% in the control animals to 33% in the virus challenged group (McGowan et al., 1993). Although the pathogenesis for embryonic death is very difficult to substantiate, infertility could well be due to ovaritis at the time of infection or due to failure of fertilisation as a result of poor quality semen from persistently infected bulls. Seronegative cows inseminated with infected semen generally fail to conceive until they develop an immune response to the virus (McClurkin et al., 1979; McGowan et al. 2003).

Abortion, which was a feature of the disease when it was first described in 1946 by Olafson and others, results if infection by NCP biotype occurs after placentation. Foetal death may be due to invasion by the virus or may be secondary to damage of the maternal placenta through disruption of its vascular nutrients. In sheep BVDV has been shown to damage the maternal vascular endothelium within 10 days of infection with a resultant placentitis that may attribute to the abortion. Much of the information on abortion has been derived from experimental infection of pregnant cows in which

abortion was shown to occur during the first trimester (Brownlie et al.. 1987; Brownlie 1990; Brownlie et al.. 2000).

BVDV also causes significant intrauterine growth retardation in many of the foetal tissues, particularly the central nervous system, musculoskeletal system and the thymus. Hypomyelination associated with cerebellar hypoplasia is the most characteristic defect seen. Other defects include thymic aplasia, brachygnathism, retinal atrophy and displasia, hydroencephaly and hydrocephalus (Bielanski and Hare 1988; Fray 2000).

An important outcome of foetal infection during the first trimester, after development of foetal membranes, but before the immune system is functional (about 45-125 days) is persistent viraemia (Houe 1994; Fray 2000). The foetus will not develop virus neutralising antibodies and the virus will be recognised as self. The foetus may be carried normally to term and be born with a persistent infection (PI) with the virus. In all recorded field and experimental data there is no evidence for persistence with the cytopathic biotype. From birth these animals are specifically immunotolerant and persistently viraemic. PI animals normally shed virus from 3-4 months of age following depletion of maternal antibodies (Palfi et al. 1993; Fray 2000), and shedding continues throughout the animal's life. The clinical appearance of PI animals ranges from normal to grossly abnormal. Many PI animals become stunted in growth and in the field they are usually recognised as "runts" in the herd. PI animals have a reduced life expectancy, with most of them surviving less than 24 months. This is due to substantial damage to the immune system and the subsequent lowered resistance to infections. Also when the foetus is infected in the second trimester the virus tend to localise and cause severe damage to the cerebral cortex and hippocampus (Hamers et al. 1998; Voges et al. 1998). Usually the lesions here are more severe and the newborn calves are depressed, with incoordination and are unlikely to survive. However, some female PI animals may survive beyond 24 months, producing PI offspring(s) before being culled or succumbing to mucosal disease (MD). In contrast to the high prevalence of BVDV in cattle populations, PI animals are only a small percentage (1-2%) of the population (Littlejohns and Horner 1990; Houe 1995; Radostits and Littlejohns 1988). Several epithelial tissues sustain BVDV replication in PI animals. BVDV antigen can be demonstrated within the keratinocytes of the tongue, skin and labia.

Infection in persistently infected cattle – Mucosal Disease (MD)

Mucosal disease was originally reported in 1953 and described as a fatal condition of cattle characterized by severe erosive lesions in the oral and intestinal mucosa (Ramsey and Chivers 1953). MD remained a mystery until the late sixties when two BVDVs – the NCP and the CP – were found in a calf with MD (McKercher et al., 1968) leading to the dual-virus hypothesis (Bolin 1995). The hypothesis states that an initial transplacental infection of the early foetus with the NCP virus results in the birth of a calf that has a lifelong persistent viraemia. These calves, and only these calves, may later develop mucosal disease as a result of superinfection with a “homologous” CP BVDV (Brownlie 2000). Mutation of the persistent NCP BVDV in the PI animal is another possible route causing the onset of MD. A MD-like syndrome was experimentally induced in sheep (Barlow et al., 1983; Gardiner et al., 1983) and following that MD was induced in cattle (Brownlie et al., 1984; Bolin et al., 1985) by infecting NCP PI animals with CP BVDV. It is postulated that immunotolerance in the PI animal allows a homologous CP biotype to freely infect, replicate and destroy the cells of lymphoid tissues and sub-epithelial connective tissues of the dermis and the gastrointestinal tract (Brownlie et al., 1984).

Mucosal disease is an invariably fatal condition, mainly of young cattle aged between 6 and 24 months. Rarely calves as young as 4 months of age, or cattle older than two years are affected (Clark et al., 1985). Clinical MD is sporadic and only a small percentage of a herd of cattle, usually less than 5%, will be affected. Occasionally, outbreaks have been observed in which up to 25% or more of calves six to ten months of age in a herd have been affected. Peak outbreaks may occur reflecting husbandry methods like mixing of cattle following weaning of beef calves or following introduction of purchased replacement stock (Radostits and Littlejohns 1988). Clinical signs include pyrexia, polypnea, tachycardia, depression, weakness and anorexia. Erosive lesions may be observed in the oral cavity, external nares and nasal cavity. Salivation often accompanies the oral lesions, which are found in 75-80% of the cases. Occasionally erosions in the coronet of the hoof may be observed with resulting lameness in some of the cases. Profuse watery diarrhoea develops 2-3 days after the onset of the disease and the faeces may contain variable amounts of fresh and or clotted blood. The animal normally dies between two days to three weeks after onset of the

disease but PI animals may also die without warning (Radostits and Littlejohns 1988). The disease has been described by Barker et al. 1993 as closely resembling rinderpest (Barker et al.. 1993).

Characteristic post mortem findings include linear shaped erosions in the upper alimentary tract, especially on the oesophagus and the omasum. Catarrhal enteritis may be observed with dark watery intestinal contents. There may also be oedema and haemorrhage of the pyloric area of the abomasum. Peyer's patches may show varying degrees of swelling and haemorrhage through out the serosa of the ileum. Histologically there are varying degrees of necrosis on the germinal centres of the lymph nodes and spleen. Oedema and inflammatory cell infiltration could be seen in varying degrees throughout the gastro-intestinal tract (Anderson 2000).

The onset of clinical disease is rapid in although some debilitating disease may occur. Chronic MD animals will show protracted weight loss, diarrhoea and progressive emaciation and an overall unthrifty appearance. Chronic lesions can be found in the mouth and on the skin generally with failure of healing of these skin lesions being a distinctive finding (Harkness et al.. 1987).

BVDV Type 2 infections

In 1993, severe outbreaks of an acute fatal syndrome of BVDV infection in dairy, beef and veal herds were reported in the USA and Canada. Fever, pneumonia, profound thrombocytopenia, haemorrhagic disease, abortions, drops in milk production, erosions of the mucosa and sudden death characterized these outbreaks (Stoffregen et al.. 2000; Alves 1996). The morbidity and mortality in these severely affected herds have been reported to be as high as 40% and 20%, respectively. The virus strains involved in the outbreaks were analysed using monoclonal and polyclonal antibodies and the polymerase chain reaction. The virus isolates from these outbreaks of severe disease were determined to be Type 2 BVDV (Carman et al.. 1998). In addition severe and fatal BVDV disease has been described in the UK following acute infection (David et al.. 1994; Hibberd and Turkington 1993), but the outbreaks have not been shown to involve BVDV Type 2. As yet there is limited evidence of spread of Type 2 BVDV beyond North America (Vilcek 2001)

Immunosuppression

BVDV may be a pivotal component in multiple-cause infectious diseases. Laboratory evidence indicates that BVDV infection causes a profound deficit in the immune response in cattle (Potgieter 1997). The immunosuppressive effect of BVDV is mainly due to the strong affinity of the virus for immunocompetent cells, which may be destroyed or functionally impaired (Potgieter 1995). At the cellular level, BVDV depresses immunoglobulin and interferon production, reduces response of lymphocytes to mitogens and impairs monocyte chemotaxis. This depression of cell-mediated immunity is accompanied by a reduced humoral immune response (Barker et al. 1993). The virus replicates in lymphocytes and macrophages in culture, while *in-vivo* there is a decrease of circulating B and T lymphocytes (Bolin et al. 1985). There is also an associated depression of polymorph nuclear cells function through a decrease in total numbers (Roth and Kaeberle 1983).

Mixed infections of BVDV and pathogens like infectious bovine rhinotracheitis virus, bovine respiratory syncytial virus, rotavirus, *Pasteurella haemolytica* have been documented to cause more severe disease. This may be due to immunosuppression as a consequence of transient leucopenia and possibly due to a neutrophil dysfunction (Howard et al. 1989; Potgieter 1995). In a study to describe the dynamics of *Neospora caninum* infection through an intensive long-term serological investigation of a large dairy herd in New Zealand, BVDV infection was found to be a cofactor for some of the abortions (Pfeiffer et al. 2000). During the epidemic of BVDV infection in Ontario in 1993, there was an increase in the number of diagnosis of abortions due to *Neospora caninum* (Alves et al. 1996).

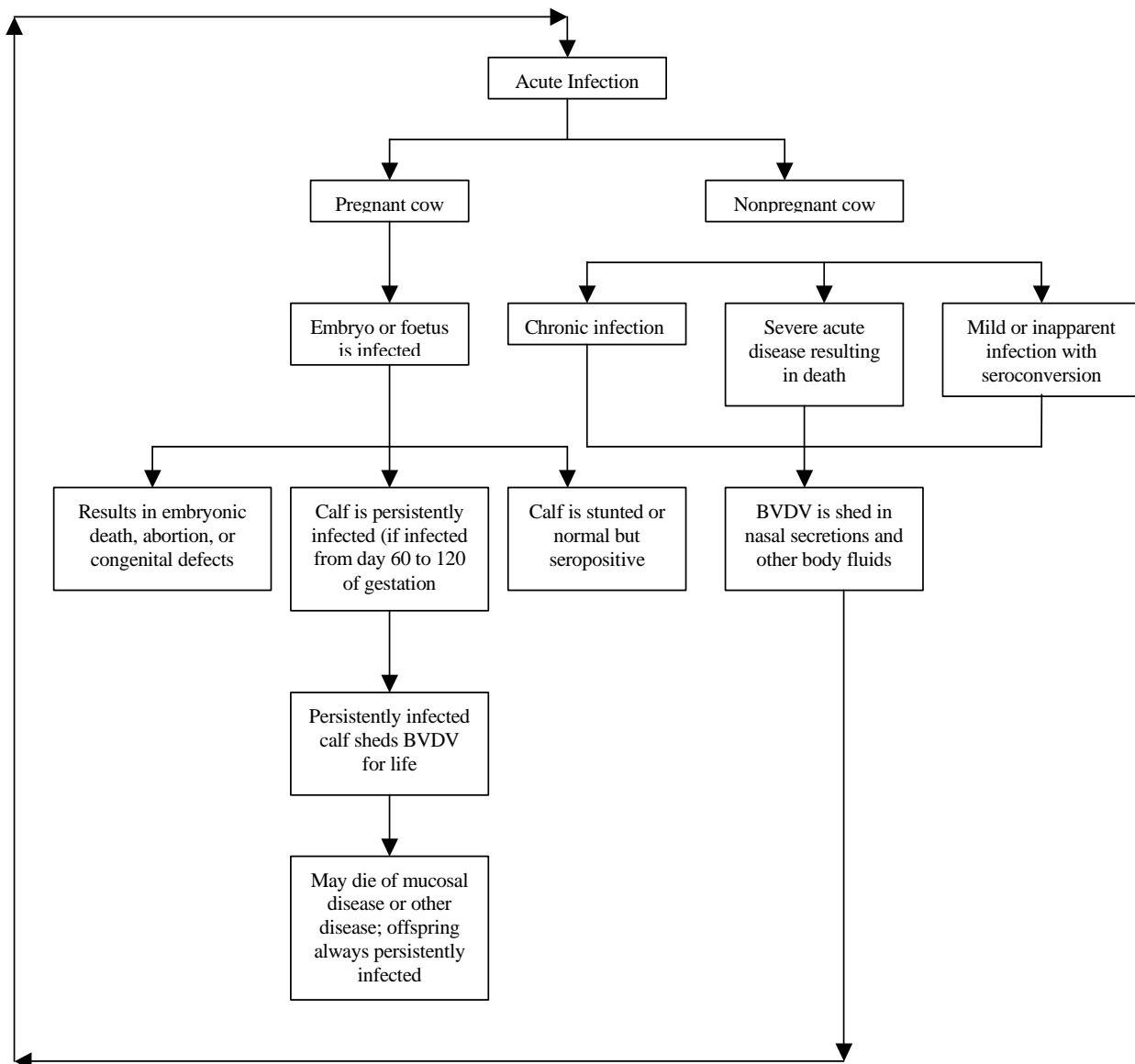


Figure 3. The clinical forms of BVDV infection. Tremblay (1996).

EPIDEMIOLOGY

Seroprevalence

BVDV infections appear to have a worldwide distribution. The prevalence of infection can be expressed in terms of antibody carriers or PI animals. The prevalence of BVDV antibody positive animals is usually between 60 and 80% with the proportion of PI animals being around 1 to 2%. The prevalence of herds with current or recent infection often ranges from 70 to 100% (Houe et al. 1994; Houe 1995). However there are some differences between regions and countries, which may be related to differences in cattle densities, housing, vaccination and management systems, as well as animal trading activities. The herd level prevalence was estimated at less than 1% in 1993 in Finland (Nuotio et al. 1999) and 30% in Norway (Loken et al. 1991). A few studies have looked at the prevalence of herds with PI animals. A survey of all cattle in 19 Danish dairy herds with unknown status of BVDV infection showed that 10 herds (53%) had PI animals in varying numbers (Houe and Meyling 1991). Another survey of 20 herds in the USA showed that 15% of the herds had PI animals (Houe 1996). Vaccination had been carried out in 15 of the 20 American herds but in none of the Danish herds. In a study carried out in Germany, 45% of 329 herds had PI animals (Frey et al. 1996).

Transmission

PI animals are considered the epidemiologically most important sources of infection excreting high amounts of virus continuously via nasal discharge, saliva, semen, urine, faeces, tears and milk. Serum samples diluted up to 10^6 may still yield the virus when examined by virus isolation techniques (Brock et al. 1991). In PI bulls, virus titres up to 10^7 have been found in semen (Paton et al. 1989). PI animals have been shown to experimentally infect 60 to 63% of susceptible animals within 24 hours where a heifer and her PI calf were put in the same yard with these animals (Littlejohns 1985; McGowan et al. 1993). However the rate of spread at pasture may be quite low, in the order of 1% (Littlejohns 1985). Acutely infected animals may excrete the virus for 3 to 10 days but the amount of virus they excrete is low compared to PI animals with very little infection to susceptible animals resulting (Brownlie et al. 1987; Niskanen et al. 1995). With the acute infection there is pyrexia, a leukopenia from about days 3 to 7

post infection and a mild nasal discharge. This is followed by a rise in antibodies, which peaks at 10 to 12 weeks post infection and immunity is assumed to last a lengthy period, possibly the animals life time (Duffell and Harkness 1985; Fredriksen et al. 1999). Due to this lifelong immunity, these animals provide protection for their foetuses against infection. In non-PI animals, maternal antibodies last for at least three months and are usually depleted at six months, but may be found in calf serum for up to nine months (Coria and McClurkin 1978).

BVDV has also been isolated from other ruminants, sheep and goats, camelids, rabbits, deer, pigs and other animals (Frolich and Streich 1998). BVDV transmission from PI cattle to a pregnant sheep resulting with a PI lamb that then transmitted BVDV to pregnant cattle had been demonstrated (Paton et al. 1997). The role of other species in BVDV infection transmission is not clear at the moment.

Transmission within herds

The natural spread of BVDV is by direct contact between animals. The main route of transmission is believed to be the oro-nasal route (Liess 1990). Infection may also spread through semen, by artificial insemination and embryo transfer, by inadequately sterilized serum products and vaccines, and also by the rectal examination glove (Brock et al. 1991; Givens et al. 2002). Iatrogenic spread through the use of hypodermic needles and nose tongs can also occur. Experimentally blood-feeding flies have been shown to carry BVDV from PI animals. The main source of infection is PI animals (Houe 1999). In a dairy herd, more than 90% of cattle will be infected within 4 to 6 months if a PI animal is introduced (Houe 1996). Following acute infection, there is transient viraemia from about 3 to 10 days and the virus is shed in all secretions such as nasal, lachrymal and urinal. However the virus shedding is at a much lower level from acute cases than from PI animals (Houe 1999).

Transmission between herds

For transmission between herds, the purchase of PI animals or a pregnant cow carrying a PI foetus constitutes the greatest risk (Houe 1999). BVDV can be transmitted from herd-to-herd by contact over the fence, use of common pastures and animal markets. Semen from PI bulls during artificial insemination and embryo transfer may also play a significant part in transmitting BVDV between herds (Houe 1999). Other species, veterinarians, herd workers and vectors such as insects are likely to play a less significant role in the transmission.

Economic losses

The losses due to BVDV infections include reduced milk production, reduced conception rates and abortions, congenital defects and growth retardation after foetal infection, respiratory disorders, other diseases and even death among animals acquiring acute infection. In addition, PI animals that result from foetal infection are often small and unthrifty, have increased susceptibility to other diseases, and frequently succumb to MD. The exact quantification of the clinical and pathological damages after infection in a population is difficult because most descriptions in the literature are based on selected clinical outbreaks and are thus not representative of the broad spectrum of the disease (Houe 1999). Only a few studies have examined the effect of BVDV in herds not selected on the basis of clinical outbreaks. Reviews of the clinical and pathological effects of BVDV infections and quantification of some of the damage have recently been published (Baker 1995; Houe 1995). An epidemiological study examining the effects of BVDV infection on the general health of cattle herds showed BVDV associated with increased risk of clinical mastitis, retained placenta, oestrus-stimulating treatments and longer calving intervals (Niskanen et al. 1995). Calculations of the economic losses are complex, and the losses in a herd from an outbreak vary according to the initial herd immunity, pregnancy status of the cow at the time of infection, and the virulence of the infecting virus strain. Accordingly, calculations of economic losses in individual herd outbreaks may vary from a few hundred to several thousand dollars (Duffel et al. 1986; Wentink and Dijkhuizen 1990; Houe et al. 1999). Estimates of economic losses from infection with highly virulent strains in a 500 cow dairy herd

have ranged from US\$40 000 to 100 000 per herd (Carman et al.. 1994; Alves et al.. 1996).

In a project to determine direct production losses and treatment due to bovine viral diarrhoea (BVD), enzootic bovine leukosis, Johne's Disease, and neosporosis in the Maritime provinces of Canada, using a partial-budget model and incorporating risk and sensitivity analyses to identify the effects of uncertainty on costs, total annual costs for an average infected 50 cow herd due to BVDV were estimated as shown below (Chi et al. 2002):

Table 2. Estimated annual cost (CDN\$) of BVDV in positive herds in Canada (50 cow herd size). Chi et al. (2002).

Effect of BVDV infection	Amount
Direct losses	
Milk yield	0
Premature culling/reduced cull value	1025
Mortality	935
Abortion and reproduction loss	406
Total direct loss	2366
Treatment costs	
Veterinary services	32.2
Medication cost	19.6
Extra labour	3.4
Total treatment costs	55.2
Total herd level costs	2422

Although some outbreaks can be devastating for the individual producer, calculations of the national losses are more relevant as a basis for deciding between control and eradication within a particular country. However, due to the variation among losses in individual herds, these calculations can only be approximated. The losses at the population level have been estimated in the range of US\$10 to 40 per calving (Bennett and Done 1986; Harkness 1987; Spedding et al. 1987; Houe et al.. 1999; Sorensen et al.. 1995).

The economic importance of a disease and the outcome of various control strategies should be evaluated in terms of the costs and benefits to society, that is social cost benefit analysis. Few such attempts have been made concerning BVDV (Bennett and

Done 1986; Spedding et al. 1987; Bennett 1987). There is also a need for sensitivity analyses of such attempts due to the many existing uncertainties, especially those regarding the distribution of strains with different virulence. There is still only moderate information regarding the number of reinfections that might occur, which is a crucial factor in order to perform cost benefit analyses. In Norway, 108 out of 3030 (3.6%) previously infected herds but released from restrictions during 1989-1996 have later been renotified (Waage et al. 1994) and in Sweden the herd level annual incidence risk was approximately 3% from 1993 to 1995 (Alenius et al. 1997). The eradication and control programme in Denmark over a 3-year period has been estimated to be US\$ 27 million (Bitsch and Ronsholt 1995). Thereafter, there will only be minor costs to the continued national surveillance. Compared with a loss of US\$ 20 million per annum, there would be a very high benefit to eradication of BVDV under the assumption that reinfection would be rare (Houe 1999).

DIAGNOSIS

Laboratory diagnosis is an important component in both the diagnosis and of BVDV infections (Brock 1995). Diagnosis based on clinical findings is difficult because the signs are usually non-specific and mild in acute infections. It is also very difficult to diagnose PI animals based on clinical observations (Houe 1996; Sandvik 1999). Only MD can be diagnosed on the basis of clinical and postmortem findings but this needs to be confirmed by isolation of CP BVDV from sites such as gastrointestinal tract (Bielefeldt-Ohmann 1995). Table 3 below shows different categories of animals in an unvaccinated bovine population where BVDV is prevalent, and results of testing for antibodies and virus in serum.

Table 3. Categories of animals in an unvaccinated bovine population where BVDV is prevalent, and results of testing for antibodies and virus in serum. Sandvik (1999).

Category	Antibody	Virus	Comments
Uninfected naïve animals	-	-	
Acutely infected animals	-	+/-	Brief and low virus titre in blood
Immune animals after acute infection	+	-	
Passively immunised calves	+	-	Antibodies detectable for 5-9 months
PI animals	-	+	
PI calves of immune dams	+	-/+	Antibodies detectable for 4-10 weeks
MD cases	-/+	+/-	Neutralising antibodies
Pregnant cows carrying PI calf	+ /+++	-	High antibody titre in late pregnancy
Immune bulls	+	-	Semen may be virus positive

Recently developed molecular techniques have provided many assays for BVDV diagnosis. Diagnosis of BVDV infection is based primarily on virus or antibody detection (Valle 2000).

Virus Detection

Virus detection is the only certain way of identifying PI animals and may be very important for all BVDV control programs without use of vaccinations. Virus can be demonstrated by isolation of infectious virus or viral antigen assays or nucleic acid detection (PCR) (Duffell and Harkness 1985).

Virus isolation

BVDV is comparatively easy to isolate in-vitro. It is commonly isolated using primary bovine foetal turbinate or testicular cells. CP strains of BVDV cause cellular vacuolation and cell death in inoculated cell cultures within 48 hours. Most commonly, BVDV isolates from field cases are noncytopathic in cell cultures. The isolated virus therefore is recognized by identifying viral antigen in positive cell cultures by immunofluorescence or immunoenzyme staining (Meyling 1984). Isolation of NCP strains generally requires 3 to 5 days of culture. The type of samples submitted for laboratory diagnosis may include serum, whole blood, nasal swabs, semen, and tissue samples. The best sample for virus isolation is from mononuclear cells in the buffy coat obtained from whole blood. Tissues from lymphoid organs such as spleen, Peyer's

patches from the small intestine, mesenteric lymph nodes and thymus are best suited for virus isolation post-mortem or from aborted foetus (Anderson 2000; Wilks 1994; Valle 2000).

When testing large numbers of samples, virus isolation is performed in microtitre plates. Viral antigen is detected using an immunoperoxidase labelled conjugate. This technique is suited to handling large numbers of samples and is easy to interpret. The assay requires 5 to 7 working days and allows for two passages to be completed. Serum, mononuclear cells, tissue homogenates are appropriate samples for the immunoperoxidase microtitre assay (Brock 1995).

Antigen Detection (Antigen ELISA)

Recently monoclonal antibody 15C5, which reacts with the E0 protein, has been shown to react broadly with most strains of BVDV and can be used to detect BVDV antigen in formalin-fixed, paraffin-embedded tissues. The epitope recognized on the E0 protein is highly conserved among BVDV isolates and is not affected much by antigenic variation. This method has research application mainly in confirming enteric, respiratory and reproductive diseases (Donis 1995).

With the availability of BVDV-specific monoclonal antibodies, several different antigen capture enzyme-linked immunosorbent assays (ELISA) have been developed (Brock 1995). The test is rapid, takes about 2 days, and is less costly as it does not involve cell cultures. ELISA methods have been widely used especially in the Nordic countries (Valle 2000). Whole blood collected in EDTA tubes from live animals is the most convenient sample for antigen detection by ELISA, and spleen and lymph nodes tissues can be used post mortem. Compared to virus isolation, ELISA has relatively low sensitivity.

Nucleic Acid detection (PCR)

Polymerase chain reaction (PCR) involves the direct detection of nucleic acids of the viral genomic RNA. This requires that the RNA be extracted from samples before detection, which is very difficult to extract RNA from samples. The ability to detect BVDV RNA by reverse transcription polymerase chain reaction (RT-PCR) amplification of cDNA has been reported by many laboratories (Brock 1995; Drew et al. 1999). This has advantages over virus isolation because of the lack of potential interference with neutralizing antibodies. Using RT-PCR amplification, BVDV can be detected up to 12 to 14 days postinfection and in most cases RT-PCR can detect from 10^1 to 10^3 -fold lower virus level than virus isolation techniques (Drew et al. 1999). Brock et al. (1991) have developed a RT-PCR bulk milk test for the detection of BVDV RNA as a screening test to identify herds infected with BVDV. The technique is comparatively costly, requires technical expertise, equipment and automation in addition to RNA extraction methods. However it is likely to be the method used in the future.

Table 4. Diagnostic tests available for detection of BVDV antigen. Brownlie (2000).

Test	Single animal or group	Requires	Measures	Interpretation
Immunoperoxidase staining	Single animal	Blood (EDTA) or tissue sample	Viral antigen	Test provides a positive or negative result
Antigen ELISA	Single animal	Blood (EDTA)	Viral antigen	Test provides a positive or negative result
Virus isolation	Single animal	Blood (EDTA)	Live virus	Test provides a positive or negative result
Bulk milk PCR	Pooled milk sample from up to 100 animals	300 ml of fresh bulk milk (no preservative added)	Viral RNA	Positive result indicates presence of at least one PI animal in milking group at the time of milking

Serology

Antibody tests indicate exposure and are useful in assessing the status of groups of animals or a whole herd prior to, or as a part of, a disease control program. There are two main options – Virus Neutralization Test (VNT) and ELISA (Sandvik 1999).

Virus Neutralization Test

The VNT is the most common serologic method used for determining levels of BVDV antibodies and it has been accepted as the reference method for BVDV tests (Edwards 1990). The test detects antibodies against glycoprotein 53 (gp 53) of BVDV. A specific virus is used at a known concentration to determine whether neutralizing antibodies to that virus are present in the sample. Virus and serum are mixed and allowed to react for an appropriate time. The mixture is then inoculated onto a susceptible cell culture monolayer to observe whether there will be a cytopathic effect (no antibody) or no cytopathic effect (antibody present). There is no universally accepted reference strain for use in this test due to the considerable antigen diversity between BVDV strains (Dobovi 1992) and differences in susceptibility depending on combinations of cell cultures and cell culture media. Due to this, the selection of the challenge virus is important for the performance of the test (Fredriksen et al. 1999). Because of the apparent lack of laboratory test standardization and possible variation in VN test results, it is important to test paired, acute, and convalescent samples simultaneously. Disadvantage of this test include taking five days, being restricted to laboratories with cell culture facilities and being sensitive to interfering factors such as BVDV antibodies (Sandvik 1999).

Antibody ELISA

Paton et al. (1997) developed a competitive ELISA to detect antibodies against protein p80/125 of BVDV. To date there are numerous variations of the ELISA tests with indirect ELISA commonly used in countries like Sweden, Finland and Norway (Valle 2000) while in Denmark a blocking ELISA is used. The advantages of ELISA are that, ELISAs are rapid to perform, relatively inexpensive and suitable for large numbers of samples. Samples suitable for the test are serum and milk. In most cases, ELISA titres correlate well with VNT titres. Using an indirect ELISA, Niskanen et al. (1989) evaluated levels of antibodies to BVDV in individual and bulk milk samples. A correlation was found between the level of antibodies in bulk tank milk samples and the presence of PI animals.

Herd-level diagnosis

There are several herd-level tests available for the determination of BVDV status and these can be used for, dividing a population or groups of animals into infected and non-infected herds, and for monitoring non infected herds. Tests can be aimed at either virus detection or antibody detection. Currently ELISA based antibody detection tests are most commonly used. They require either bulk milk samples (Niskane et al.. 1991; Bitsch 1997), or serum or milk from a small number of animals of a certain age group, generally referred to as spot tests (Houe 1992; Houe 1994; Lindberg 1996; Sandvik 1999).

The theoretical basis for bulk milk tests for antibodies to BVDV is that herds with current infection will usually exhibit high levels of antibodies to BVDV in bulk milk (Niskanen et al.. 1991; Bitsch and Ronsholt 1995; Drew et al.. 1999). This is also same for recently recovered herds due to the presence of long lasting antibodies. Bulk milk is easy to obtain, represents the whole milking herd, and comparatively the cost of sampling and analysis is substantially lower. The main disadvantage is that it is limited mainly to non-vaccinating dairy herds only (Niskanen et al.. 1991).

Through testing bulk tank milk samples from 123 Swedish herds, Niskanen (1993) described a positive relationship between the prevalence of antibody positive cows in a

herd and the level of antibodies to the virus in the bulk tank milk, as determined by an indirect ELISA. Individual milk samples and blood samples were also tested in this study. In 32 herds with an absorbance value of 0.20 or less there were very few or no antibody positive cows found, where as in 29 herds with an absorbance value of at least 0.81, 87% to 100% of lactating cows were antibody positive to BVDV. The remaining 62 herds with an absorbance range of 0.2 to 0.8 had on average 19% to 90% antibody positive lactating cows.

The theoretical basis for spot tests is the high probability of seropositivity in groups of calves where PI animals are present, due to the efficient transmission of the virus from PI individuals to the surrounding stock (Houe 1994). In general, all animals born after the removal of the last PI animal will presumably be seronegative, except for calves born with antibodies resulting from an in-utero infection in late gestation shortly before the last PI animal was culled. Thus, detection of antibody-positive individuals in a spot test directed towards young animals is an indication of current infection. If a spot test does not detect presence of antibodies, it may well be assumed that the herd is not infected.

Spot tests however are more labour intensive, time consuming and more expensive than bulk milk tests.

Houe (1995) studied the reliability of serological analysis of ten young stock for BVDV antibodies as a means of screening for BVDV status in dairy herds. Bulk tank milk as well as blood samples from ten young stock aged 8-18 months from 42 Danish dairy herds. The herds were divided into two groups. The first group (24 herds) had three or less antibody carriers and only nine (4%) of the 240 young animals tested were antibody positive. The second group (18 herds) had eight or more antibody carriers and 172(96%) of the 180 young animals tested were antibody positive. Bulk milk titres were generally found to be higher in the second group and a spearman correlation coefficient of 0.77 was found between number of antibody positive animals and the antibody titre in the farm's bulk tank milk.

Brock (1995) developed a PCR bulk milk test for the detection of BVDV RNA as a screening test to identify infected herds. Over a three-year period, Renshaw et al.

(2000) tested 144 samples from 97 farms for BVDV using an RT-PCR assay in conjunction with a classical virus isolation (VI) procedure to measure the relative effectiveness of the techniques. Virus was detected with both methods when milk from a PI animal was diluted 1:600 with the milk from a herd of BVDV negative animals. The cross relation between the two assays was 95.9%. In terms of sensitivity, specificity and turnaround time, RT-PCR was superior, detecting 7 of the 17 VI positive samples as negative and 7 VI negative samples as positive. RT-PCR however may not detect naturally occurring BVDV isolates with minor sequence variations in the primer regions. Factors such as cost, technical expertise, equipment and automation, remain of consideration compared with other methods but with time this could be the method of the future.

CONTROL OPTIONS

There are three main control options for BVDV infection:

- Vaccination using commercially available vaccines
- Controlled natural infection of non-pregnant animals by maintaining PI animals in herds
- Eradication by identification and removal of PI animals.

Vaccination

Many vaccines (modified and live) have been developed and are widely available on the market following Coggins et al.. (1961) description of an attenuated BVDV strain. Initially the vaccines were aimed at reducing postnatal infections. However, nowadays postnatal infections only run a very mild course. The increased understanding of the pathogenesis of the disease mainly in the 1980s demonstrated that PI cattle are the greatest sources of transmission of BVDV and often develop MD leading to high economic losses. This led to the recognition that BVDV vaccines should primarily focus on the prevention of infection of the foetus (Lindberg and Alenius 1996). The recent emergence of more virulent strains of BVDV in Canada and the USA has increased the need to protect cattle against postnatal infection.

Types of BVD vaccines

Modified Live Vaccines

Modified live vaccines contain attenuated strains of BVDV that replicates in the host to elicit an immune response (Bolin 1995). Many modified live vaccines contain the CP strains Oregon C24V, NADL or Singer (Phillips et al. 1975). Attenuation may have been achieved by multiple passaging BVDV strains in cell cultures from bovine and/or non-bovine origin. A temperature sensitive mutant is selected by treating BVDV with nitrous acid, which is then followed by limiting dilutions at restrictive and permissive temperatures to give a mutant that forms the basis for production of a vaccine. It has been very difficult to ascertain whether these strains are really attenuated because there have been no studies comparing the virulence of the parent and vaccine strains. In addition the molecular basis for attenuation is poorly defined (Vilcek et al. 1998).

Inactivated or Killed Vaccines

For the development of killed vaccines, BVDV strains are grown to high titres and then rendered non-infectious, usually by treatment with chemicals. Currently the most widely used chemical is ethyleneimine. The resultant vaccines contain intact killed virus, which is then formulated in an adjuvant to induce adequate immunity. BVDV vaccines are usually administered per intramuscular or subcutaneous injection (Carman et al. 1998).

Combined Vaccines

BVDV, possibly through suppression of the immune system of the host, is often involved in the pathogenesis of respiratory diseases without inducing the disease on its own. For this reason, BVDV vaccine strains are often included in multivalent vaccines to prevent respiratory diseases. Other microorganisms, which may be included, are bovine herpesvirus 1, bovine parainfluenza-3 virus, bovine respiratory syncytial virus and *Pasteur Ella* and *Haemophilic* species (Littlejohns 1985).

Disadvantages of vaccines

(i) *Viral diversity*

The very large antigenic and genetic diversity of BVDV strains is well documented and lead to differentiation of classical BVDV 1 from the more recently recognized BVDV 2 strains. The BVDV 1 strain is subdivided from 1a to 1j based on nucleotide differences. An important question is whether vaccination with a strain of one antigenic group induces protective immunity against strains of other antigenic groups. There have been reports of neutralizing antibodies produced against 20 different strains by vaccinated calves (Bolin and Rid path 1989; 1990). However the antigenic variation between these strains was unknown. It is clear though that more research is needed to establish the impact of antigenic diversity on vaccine development.

(ii) *Efficacy of BVDV vaccines*

Various experiments have been performed to evaluate the efficacy of live and killed BVDV vaccines. Only one vaccine protected all vaccinated cattle from transmitting the challenge virus to their foetus (Brownlie et al. 1995). However no vaccine gave complete protection and thus there is room for improve their efficacy. Even live vaccines did not protect all cattle from congenital infection. Some studies also reported no correlation between neutralizing antibody titres and protection from congenital infection (Zimmer et al. 1996; Brusckke et al. 1997).

The overall low to moderate efficacy of BVDV vaccines in preventing congenital infection may be partly explained by the mode of challenge, which may be too heavy. High titres of challenge virus are usually inoculated intranasally.

Vaccination of PI cattle when super-infected with a CP strain failed to protect against development of MD (Bolin et al. 1985). There are few well-designed studies to demonstrate that BVDV vaccines can protect cattle from clinical signs and reduce or prevent virus replication in vaccinated cattle. In some studies, however, vaccines provided complete protection against the virus (Howard et al. 1994; Bolin and Rid path 1996). Calves administered a multivalent vaccine containing an attenuated BVDV 1

strain were clinically protected against challenge with a BVDV 2 strain, where all unvaccinated calves were euthanised due to severe disease (Cortese et al. 1996). Hence existing live vaccines containing BVDV 1 strains may be used to prevent severe disease caused by BVDV 2 infections. Transmission experiments to examine whether BVDV vaccines are able to reduce the transmission of challenge virus among cattle have not yet been performed.

(iii) *Safety*

Live BVDV vaccines have been associated with adverse reactions. These include, a condition resembling MD observed in cattle shortly after vaccination (Chennekatu et al. 1967), crossing the placenta and infecting the foetus resulting in mild or severe clinical signs (Liess et al. 1984) and also being immunosuppressive (Roth and Kaeberle 1983). Another disadvantage of live vaccines is their possible contamination with extraneous agents mainly NCP BVDV (Nuttall et al. 1977).

Killed BVDV vaccines have less disadvantages except that they may induce a local reaction at the site of injection depending on the type adjuvant used. They may also have residual infectivity if inactivation was not performed correctly and are very expensive and laborious to produce.

Although vaccination has been used for decades the disease is still widespread. This, in addition to the problems listed above makes a poor argument for the use of vaccines to protect against BVDV.

Controlled Natural Infection

It is possible to use known PI animal to infect susceptible cattle (Horner 1996). Acute infection of susceptible cattle will lead to lengthy immunity (Kahrs et al. 1966; Duffell and Harkness 1985; Fredriksen et al. 1999). Exposure could naturally occur from around six months, when maternal antibodies wane, up to the time of insemination. Putting animals in the same yard increases the rate of transmission, compared to grazing the animals together (Littlejohns 1985).

There are many disadvantages associated with this regime. It is very difficult to determine the time of infection, or prove infection occurred at all (Moerman et al. 1993). There are reports of prolonged shedding after infection, raising the undesirable possibility of virus transmission during pregnancy (Meyling et al. 1990; Moerman et al. 1993). Animals exposed to the virus are also susceptible to other infections in the viraemic phase due to immunosuppression, possibly leading to increased respiratory and enteric problems (Potgieter 1995; Howard et al. 1989; Larsson et al. 1994; Taylor et al. 1997). PI animals are also likely to develop MD before 24 months of age, economic losses and distress associated with the disease (Houe et al. 1993; Baker 1995). The use of controlled natural infection probably has several considerable drawbacks.

Eradication by identification and removal of PI animals

Eradication refers to the identification and removal of PI animals (Harknes 1987) from the herd. It is based on the understanding that, (1) in practice, a herd is not infected until one or more persistent infections have been established; (2) the high incidence of self clearance of the disease will reduce the prevalence of BVDV infections in a cattle population even without active disease control, provided the virus is not re-introduced; and (3) BVDV cannot persist within a herd if there is absolutely no contact between PI animals and susceptible animals in early pregnancy. Understanding of the above facts, supplemented by the knowledge of the herd structure and important risk factors for between herd transmission present in the area/country in question are the basis for BVDV eradication (Lindberg and Alenius 1999).

Presently, there are many tools available for herd-level diagnosis (Niskanen et al. 1991; Niskanen 1993; Houe 1992; Houe 1994). These coupled with improved methods for individual diagnosis play an important role in eradication. Eradication relies upon a primary herd level step where infected herds are identified, followed by removal of PI animals from these herds as a second step (Bitsch and Ronsholt 1995). Another extremely important step (Lindberg and Alenius 1999) is the implementation of a farm biosecurity measure aimed at protecting herds against BVDV infection. Thus a general outline for BVDV eradication from a population of herds includes three steps: -

- (1) Division of the population into infected and non-infected herds
- (2) Monitoring of non-infected herds and
- (3) Virus clearance in infected herds

Determination of BVDV status

There are several herd-level tests available for the determination of BVDV status. These can be used for the definition and monitoring of herd infection status. Tests can be aimed at either virus detection or antibody detection. Currently mainly ELISA based antibody detection tests are being used either on bulk milk samples (Niskanen et al., 1991; Bitsch 1997) or on serum or milk from a small number of animals of a certain age group (spot tests) (Houe 1992; Houe 1994; Lindberg 1996). The herd status should be well communicated to farmers and the role of farmers in eradication should not be underestimated.

Monitoring of non-infected herds

The purpose of monitoring is to identify herds with low risk of infection mainly for trade purposes but also to motivate farmers if BVDV free cattle can be traded at higher prices. In Sweden, monitoring is based on (1) a testing procedure to prove absence from BVDV infection, (2) repeated sampling with consistently low antibody or antibody-negative test results, at herd level, (3) biosecurity measures actively maintained by the farmer (Lindberg and Alenius 1999). Bulk milk testing is a sensitive method for monitoring the BVDV status in non-infected herds. The basis is that, a large proportion of the herd will be seronegative and hence bulk milk will contain low or very undetectable levels of antibodies (Niskanen 1993; Junitti et al. 1987). In such herds a bulk milk test can detect a new infection at a very early stage, often even before the birth of the first PI animal. In other words, a bulk milk test can reveal the presence of a single seropositive animal within an otherwise seronegative herd even in large herds. Farmers whose herds are monitored on bulk milk should be encouraged to avoid buying seropositive replacement stock as this may increase antibody levels in the bulk milk to the point where infection status of the herd is reclassified.

Virus clearance in infected herds

The aim of virus clearance is to have a lasting improvement in animal health and to minimise economic losses due to BVDV infections. It is based on identification and removal of all PI animals including unborn calves at the initiation of the process. A

systematic and reliable strategy based on disease characteristics at the herd level is necessary to enable the cost and time efficient intervention. A protocol for virus clearance includes (1) an initial test on all animals, (2) a subsequent follow up on calves born and (3) a follow-up on those dams, which were seronegative in the initial screening (Littlejohns 1985). Farmers with infected herds should be advised to refrain from selling animals to reduce transmission to other herds.

In the initial herd investigation, all animals are tested for BVDV. This will identify PI animals and non-immune dams. Non-immune dams are the only group able to carry PI foetuses causing persistent infection. Based on the results, PI animals are culled, dams suspected to be carrying PI foetuses are quarantined and susceptible pregnant animals are prevented from coming in contact with PI animals.

Approximately one year after initial screening, all calves born are tested for both antibody and virus to identify what were PI foetuses. In some cases, testing calves before their first colostrum will be a quick way of detecting PI calves. PI calves are then culled.

Following testing of newborn calves, all previously seronegative cows of reproductive age should be retested. Retesting is particularly important if infection status of dams' calves was PI or unknown. Only antibody detection is necessary. Cows should be retested if they were initially negative.

Usually the prospect and prognosis of fast disease clearance is very good in herds where BVDV has been circulating for a moderate or long period. In newly infected herds the challenge of disease clearance increases with herd size. If a single or a few cows are suspected to be carrying PI calves, they can be identified and quarantined and their calves then tested and culled. However if there are a lot of PI foetuses and a large number of susceptible cows, immediate removal may be detrimental as each PI calf represents the risk of a disease outbreak. Here vaccinating all animals with an efficient and safe inactivated vaccine to prevent foetal infection may be valuable (Lindberg and Alenius 1999).

Experiences of BVDV Eradication

BVDV eradication based on the detection and culling of PI animals has been carried out across many European countries without the use of vaccines. Eradication was initiated in Sweden and Norway in 1993 and Denmark and Finland 1994 (Lindberg and Alenius 1999). Other countries involved in eradication are Italy (Rome province), Slovenia, initiated in 1994, and Shetland Islands, which also initiated eradication in 1994 (Synge et al.. 1999).

Norway

In 1994 a nation wide eradication programme was started following an agreement between the cattle industry and the Ministry of Agriculture (Waage et al.. 1994). Over the eradication period, the herd prevalence of BVDV positive young stock dropped from 10% to 0.7% in dairy herds and from 20% to 8% in beef herds (Valle 2000). A distinctive feature of the Norway BVDV programme is the use of regulatory means to impose movement restrictions and isolate premises suspected of infection (Lindberg and Alenius 1999). BVD is a notifiable disease, which makes it easy to impose movement restrictions (Bitsch and Ronsholt 1995).

Sweden

Eradication was initiated in 1993 at the request of farmers using a voluntary programme with farmers bearing the cost of sampling, testing and eradication. In 1993, 23% of the 18,202 herds tested were found to be BVDV naïve (Forshell et al.. 1994). In 1997 55% of the dairy herds were declared negative (Hult 1997). Trading of untested animals was the main problem with purchased PI animals infecting seronegative herds. Where possible farmers were encouraged to buy animals from BVDV negative herds.

Finland

A comprehensive co-operatively funded eradication programme was launched in 1994. The prevalence in beef breeding units was 30.2% in 1993 and in dairy herds it was 3% (Niskanen 1993). The general prevalence has since dropped to 0.8-1.6% in 1997 (Nuotio et al.. 1999). The success of the programme is due to a low level of trade between herds, the structure of husbandry with small isolated herds and strict control of live animals movement, which existed until 1995 (Nuotio et al.. 1999).

Shetland Islands

A scheme to control and eradicate BVDV was initiated in 1994 by local veterinary surgeons and funded by the Shetland Islands Council and Shetland Enterprise Company. A total of 6150 animals were tested from 213 herds and 43% of herds were found to be BVDV naïve. The scheme was stopped in 1997 when all persistent excretors had been removed. The major risk to the Shetland Islands is from imported stock, especially where cows are in calf. It is therefore important to have import restrictions or test all in calf cows on arrival and test their calves after birth (Synge et al., 1999).

BVD/MD IN NEW ZEALAND

Epidemiology

The disease was first suspected in New Zealand around 1961 and although CP agents were isolated from an animal presenting with MD-like syndrome, the case was not confirmed as BVD and prominent kidney lesions suggested a different diagnosis (Salisbury et al. 1961). In 1966, Fastier and Hansen (1966) conducted a serological survey on herds, which exhibited present or past history of upper respiratory tract disease, for presumptive evidence of BVD. Forty-one (41%) of 118 cows tested had diagnostically significant antibody titres, but virus isolation was unsuccessful. A group of 60 animals, in which respiratory disease was not a factor, were also tested and 25% had significant antibody titres. A case of MD with virus isolation was reported in a Jersey heifer the following year, confirming the presence of the disease in New Zealand (Jolly et al. 1967).

A study done in Otago in 1971 by A.J. Robinson showed 66% cattle were antibody positive and 28% of sheep were positive in the South Island. In this study serum was collected from two local abattoirs to record the occurrence of serum neutralising antibodies to BVDV. In 1975, examination of laboratory records of diagnostic sera submitted over an 18-month period from most areas of New Zealand revealed that 34% possessed neutralising antibodies to BVDV (Durham and Burgess 1977). This confirmed the wide distribution of BVDV infection in New Zealand. In 1990, Littlejohns and Horner reported a 60% prevalence of BVDV infection in New Zealand

based on testing of diagnostic sera submitted to Central Animal Health Laboratory. Similarly Perez reported prevalence of approximately 60% in beef herds from studies done in 1994 and 1995 (Perez 1994; Perez 1995). In the 1995 study, 3 dairy-beef units and 3 dairy-calf units were selected from the Manawatu region to examine the prevalence of BVDV antibody positive animals. Animals were tested at 24 weeks, 6 months and 1 year of age. The result varied from 0% at 1 year old to >90% at 24 weeks age (Perez 1994; 1995).

Clinical syndromes

BVDV infections have been associated with a wide range of clinical entities in New Zealand. Mossman and Hanley (1976) associated the disease with foot lesions and polyarthritis. A high incidence of congenital defects and stillborn calves over a four-year period in a large beef herd was attributed to BVDV infection (Gill 1989). Littlejohns and Horner (1990) reported enteritis in autumn and winter, pneumonia, concurrent parasitism and bacterial infections such as *Dermatophilus* and *Salmonella* to be associated with the disease. Vermunt and Bruce documented a case of illthrift in 1993 and pestivirus is now one of the main reported causes of illthrift and diarrhoea in cattle in New Zealand.

Motha et al. (1997), reported serological evidence indicating BVDV as one of the viruses responsible for bovine respiratory disease. Bovine pestivirus has recently been described as an important cause of reproductive failure in heifers (Pickering 1999).

Diagnosis

Comprehensive laboratory facilities are available in New Zealand for the investigation of BVD virus infection in cattle (Wilks et al. 1994). Both virus neutralisation test, which detects antibodies from serum, and immunoperoxidase test, which detects viable virus, are available at the National Centre for Disease Investigation (NCDI). These tests require use of a cell culture system. Also available at the NCDI, Regional Animal Health Laboratories and commercial veterinary laboratories are antibody and antigen ELISA tests. The tests can be used for single animal diagnosis or for herd level screening.

Control

From the original CP virus isolated by Jolly et al. in 1967, a live attenuated vaccine – Bovax TVL, was manufactured and sold throughout the 1970's before being withdrawn due to low sale volumes (Wakelin 1993). According to Wakelin 1993, Imported inactivated vaccines became available after Bovax, these were only provisionally licensed due to very minimal available information relating to their efficacy. The situation remains the same today; the issue of antigenic variation poses a question about their efficacy, their duration of protection is unknown, and their ability to protect the foetus has not been tested.

Artificial Insemination centres in New Zealand are required to screen all bulls for BVDV before entry, quarantine after entry and test the semen prior to artificial breeding (Voges et al. 1998).

The exact extent of losses due to BVDV infection in New Zealand is not known, but on a national scale is likely to have a significant cost to the cattle industry (Anderson 2000).

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LIST OF ABBREVIATIONS

BDV	Border Disease Virus
BVD	Bovine Virus diarrhoea virus
BVDV	Bovine virus diarrhoea virus
CP	Cytopathic
CSFV	Classical Swine Fever Virus
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
MD	Mucosal disease
NCP	Non-cytopathic
PCR	Polymerase chain reaction
PI	Persistent infection
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
UK	United Kingdom
USA	United States of America
VNT	Virus Neutralisation Test

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Paper I

G Thobokwe, C Heuer . Infrequent abortions are also associated with bovine viral diarrhoea virus (BVDV) and *Neospora caninum*, *New Zealand Veterinary Journal*, Accepted (October 2002)

**Infrequent abortions are also associated with bovine viral diarrhoea virus (BVDV)
and *Neospora caninum***

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Abstract

AIM: To estimate the distribution and causes of abortion in dairy herds across New Zealand during the 2001/2002 breeding season.

METHODS: A questionnaire survey was sent to all veterinary practices that were listed as members of the New Zealand Veterinary Association. Veterinarians were requested to indicate the numbers of dairy herds where the rate of aborting cows was None (0%), Low (1-5%) and High (>5%). They were also asked to indicate all laboratory submissions and the specimens, tests and results for each category.

RESULTS: Forty-two large animal practices serving 1,431 dairy clients from all except one region in New Zealand responded. A total of 58 (4%) herds had >5% abortions in the 2001/02 season and most laboratory submissions were from this category. The main causes of abortion among a total of 52 herd submissions were *Neospora caninum* (36.5%) and bovine viral diarrhoea virus (BVDV) (17.3%). This frequency distribution was similar in 35 herds submissions from the Low (1 to 5%) abortion category in that 34% were due to *Neospora caninum* and 15% were due to BVDV.

CONCLUSION: BVDV and *Neospora caninum* are the most frequently diagnosed causes of abortion in dairy herds New Zealand with a problem of abortion as well as in herds with a low level of abortion.

KEY WORDS: *Abortion, bovine viral diarrhoea virus, Neospora caninum*

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ABBREVIATIONS: BVDV bovine virus diarrhoea virus, CP cytopathic, NCP non-cytopathic, MD mucosal disease, PI persistently infected

Introduction

Bovine viral diarrhoea virus is a major reproductive pathogen in cattle populations worldwide with prevalence of infection in a population often in excess of 70%. Two species of BVDV have been genetically classified, one as BVDV-1 and the other BVDV-2. BVDV-1 viruses have at least 10 serologically distinct subspecies (1a-1j) and have a worldwide distribution. BVDV-2 viruses are as yet largely restricted to the United States of America and Canada. BVDV also exists as two distinct biotypes, cytopathic (CP) or non-cytopathic (NCP). The NCP biotype persists and circulates in cattle populations. It is usually involved in various manifestations of the disease. The CP biotype, either through mutation of or superinfection with the homologous NCP can cause mucosal disease (MD) in persistently infected (PI) calves. In MD the animal becomes immunotolerant to the CP biotype with resulting pathological consequences such as profuse watery diarrhoea, progressive emaciation, stunted growth and death (Brownlie et al 2000).

BVDV infections can result in a wide range of clinical syndromes ranging from mild inapparent clinical signs to fatal mucosal disease. Of particular importance is infection in pregnant cattle and is the main focus of this paper.

The foetus is vulnerable to infection when susceptible animals come into contact with BVDV. BVDV infection of the dam during the pre-implantation period may result in a high incidence of embryological or foetal mortality, resulting in repeat breeders. Infections which occur between implantation and the end of the fourth month of pregnancy (approximately days 40 to 125) are characterised by foetal death, abortion, mummification, the birth of PI calves or a limited degree of teratogenesis (Moening and Leiss 1995). PI animals are considered the epidemiologically most important sources of infection excreting high amounts of virus continuously via nasal discharge, saliva, semen, urine, faeces, tears and milk (Fray et al 2000).

The protozoan parasite *Neospora caninum* is one of the most important infectious agents causing abortion in cattle worldwide (Dubey 1999). Previously *Neospora caninum* has been associated with abortion outbreaks (Reichel 2000). Since the dog has been defined as a definitive host for *N. caninum*, several reports have putatively linked abortion storms on dairy farms to horizontal transmission of this parasite by oocyst-contaminated cattle food (Anderson et al 1995). Thus, the presence of dogs on a farm increases the risk of abortions in cattle due to *N. caninum* (Bartels et al 1999). The most important feature of this disease is that it can be maintained in cattle as chronic infection, which can be passed on to the foetus during pregnancy. Vertical transmission through generations of cattle appears to be the method through which infection is maintained in herds. In endemic herds, the majority of calves born to seropositive cows have serologic evidence of congenital infection and the rate of seropositivity is similar among calves and adult cattle in the herd (Anderson et al 2000.) In a study to describe the dynamics of *N. caninum* infection through an intensive long-term serological investigation of a large dairy herd in New Zealand, heifers and dams born from cows, which tested at least once positive, were found to be 2.2 times as likely to abort as those that always tested negative (Pfeiffer et al 2000).

Cattle infected with *N. caninum* develop humoral and cellular immune responses (Hemphill and Gottstein 2000). However, in contrast to the closely related parasite *Toxoplasma gondii*, immunity to *N. caninum* does not protect against recrudescence during gestation and thus diaplacental transmission of the parasite to the foetus. The incidence of abortion was assessed in seropositive cows, and a 3.5 times higher abortion risk compared to seronegative cows was found (Davison et al 1999).

This paper outlines the results of a questionnaire survey sent to veterinary practices in New Zealand to assess the proportion of abortion in cattle due to BVDV or *N. caninum*.

Materials and Methods

In October 2001 a questionnaire was mailed to all veterinary practices listed as members of the New Zealand Veterinary Association. Veterinarians were requested to indicate the location of the practice at an administrative regional level, and the number of dairy farms that were listed as their client during the 2001 breeding season. Each dairy herd was to be allocated one of three categories of abortion frequency: None (0%), Low (1-5%) or High (>5%). For one or more specimens collected from a herd and submitted to a laboratory for diagnosis of abortion, the practices were asked to indicate the abortion percentage of the herd, the month the sample was taken, the specimen type and number, laboratory test(s) performed and the reported result(s).

Results and Discussion

Among 50 large animal practices that responded, 8 (16%) had too little information and were therefore excluded from the analysis. The 42 remaining practices covered 14 of the 15 administrative regions of New Zealand and a total of 1,431 out of a possible 13,861 dairy herds with an average herd size of 217. The proportions of herds sampled from each region are shown in Figure 1. Regions with a large population of dairy herds were well represented and the overall sampling fraction was roughly 10% of the entire population. Hence the distribution of abortions shown by this study may represent the New Zealand situation fairly well. An overall Chi-square analysis showed no correlation between region and abortion categories ($p=0.0000$).

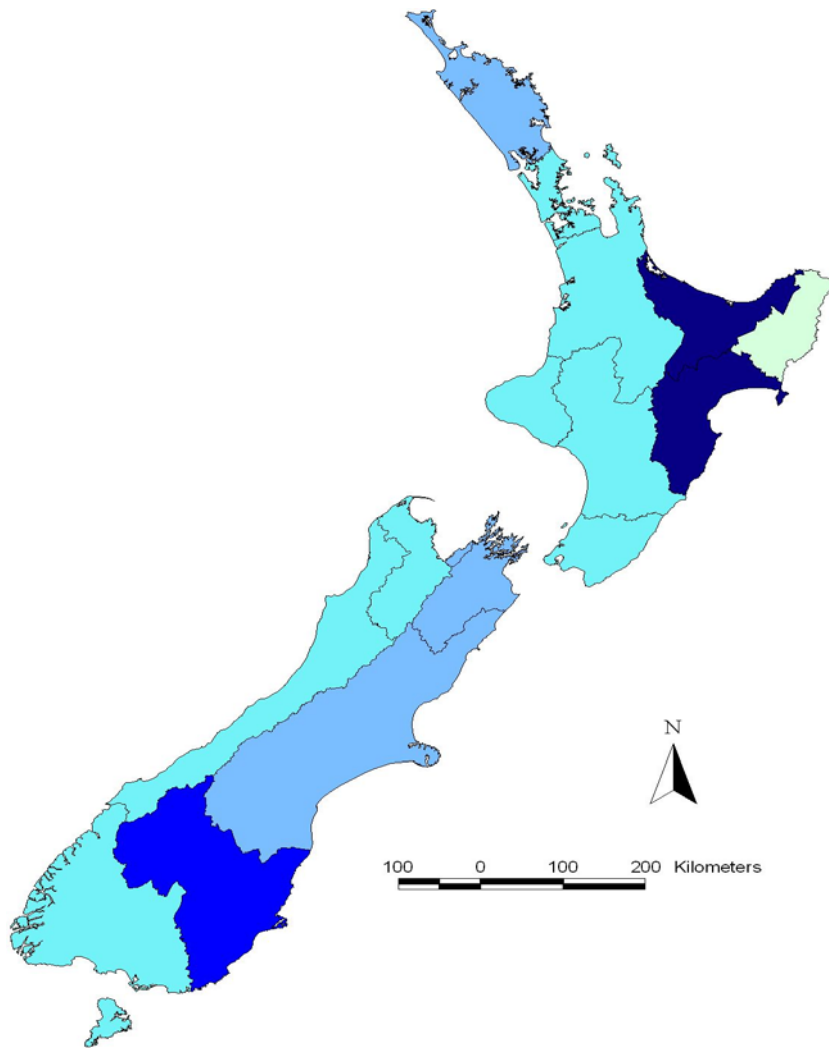


Figure 1. The proportion of all herds present in a region of a total of 1,431 dairy herds in 14 administrative regions in New Zealand, which responded to an abortion survey in cattle.

0% 1-5% 5-21% 22-39% 40-65%

The breakdown of abortion at the herd level in the three categories is shown in Table 1. Fifty-eight (4%) of all herds had High abortion frequencies (> 5%). Thus these may be regarded as herds with a major problem of abortion during the 2001/02 breeding season.

Table 1. Origin of herds and their abortion distribution.

Region	Total number Of Herds	Level of Abortion		
		None (0%)	Low (1 to 5%)	High (>6%)
Auckland*	22	1	21	0
Bay of Plenty	461	147	283	31
Canterbury	94	47	45	2
Gisborne*	0	0	0	0
Hawkes Bay*	42	38	3	1
Manawatu-Wanganui	9	9	0	0
Nelson-Marlborough	71	51	18	2
Northland	232	7	216	9
Otago	120	58	59	3
Southland*	4		3	1
Taranaki	111	12	92	7
Waikato	235	119	114	2
Wellington*	14	5	9	0
West coast*	16	3	13	0
TOTALS (%)	1431	497(34.7)	876(61.2)	58(4.1)

* Excluded in the chi-square analysis.

About 203 samples from 52 (6%) of 934 of the herds reporting abortions were submitted for laboratory investigation. The samples submitted constituted blood, serum, foetus, placenta, stillborn calves in varying numbers and variety per case. Results indicated that 17.3% of all abortions were due to BVDV, 36.5% were due to *N. caninum*, and 21.2% could not be associated with either BVDV or *Neospora* due to either inconclusive titre levels or non-specific histopathological findings. Other causes of abortion included mycotoxins, *Salmonella* spp, urea and plant poisonings (17.3%). In only 7.7% of all abortions investigated could the cause not be established (Figure 2). Most diagnoses of BVDV (67%) were based on the submission of a foetus or foetal material, stillborn calves and placenta. A serum sample on its own is often not

conclusive, as a high maternal antibody titre does not always indicate the specific cause of abortion, due to the complex pathogenesis of the disease. In contrast, high antibody titres against *N. caninum* associated with abortion are usually indicative of *Neospora* abortion. *N. caninum* was established as the cause of abortion based on serum antibody titres in 57% of cases and on histo-pathological changes in 43% of cases.

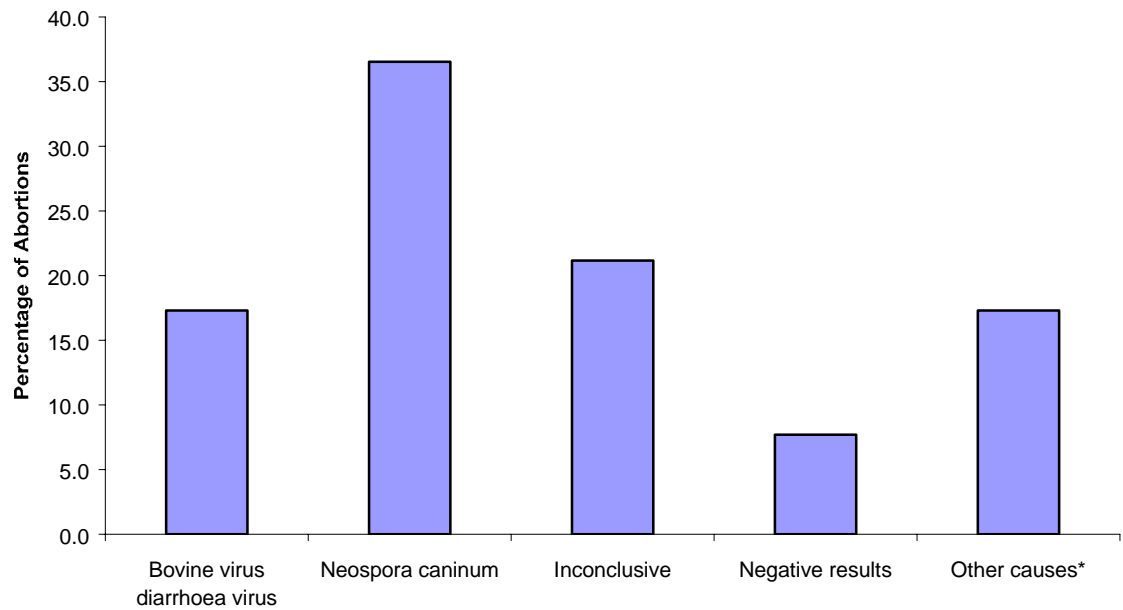


Figure 2. Distribution of diagnosed causes of abortion from 52 herds, which had submitted samples (*Salmonella spp, mycotoxins, urea and plant poisoning)

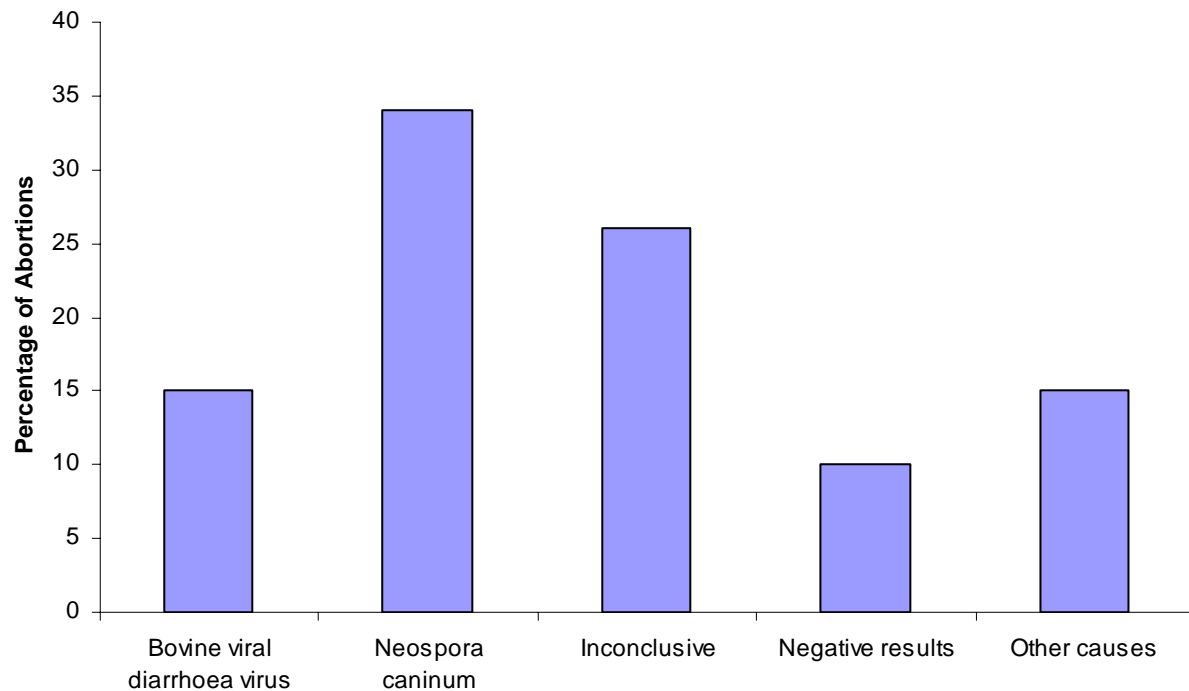


Figure 3. Distribution of diagnosed causes of abortion from 35 herds with low abortion frequency (1-5%) that submitted samples (other causes include *Salmonella* spp, mycotoxins, urea and plant poisoning).

The rate of submission of samples increased with the increasing percentage of abortion in a herd. However, since most (876) of the aborting herds were in the low abortion category, most of the herd samples (35/52 herds) were from this category. Among these herds with a low level of abortion, 15% abortions were due to BVDV, 34% due to *N. caninum*, 26% inconclusive, 15% other causes and 10% were of an unknown cause (Figure 3). This shows that BVDV and *N. caninum* were the most frequently diagnosed causes of abortion in dairy herds in New Zealand with or without a problem of abortion.

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We would like to acknowledge the time the veterinary practices spent completing and returning the questionnaires.

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Paper II

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Validation of a bulk tank milk antibody ELISA test to detect active infection with bovine viral diarrhoea virus (BVDV) in New Zealand Dairy Herds

G Thobokwe*[§], C Heuer* and D Hayes[†]

Abstract

AIM: The aim of this study was to assess the relationship between results of a bulk tank milk antibody ELISA for bovine viral diarrhoea virus (BVDV) and the prevalence of BVDV sero-positive young stock as an indication of active infection of the herd.

METHODS: Bulk tank milk samples from 724 dairy herds in the Waikato, Bay of Plenty and Northland regions of New Zealand were tested for BVDV antibodies. From this random population subset, 20 herds were again randomly selected from each of the quartiles of the percentage sero-positive (%SP) ELISA result and contacted for blood sampling. ELISA antibody test results on blood of 15 calves aged 6 – 17 months from each of 50 herds were available for final analysis.

RESULTS: Based on the blood results, 34 herds were classified as non-infected (0-3 sero-positive among 15 calves) and 16 herds as infected (5-15 sero-positive among 15 calves). Receiver operator characteristic (ROC) analysis suggested an optimal cut-off for bulk tank milk of 80 %SP yielding 81.2% sensitivity and 91.2% specificity.

CONCLUSION: We concluded that bulk tank milk can be used to determine the BVDV infection status of dairy herds in New Zealand.

KEYWORDS: Bovine viral diarrhoea virus (BVDV), dairy herds, Bulk tank milk, ELISA, young stock, seropositivity.

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Introduction

Bovine viral diarrhoea virus (BVDV) is a common pathogen in cattle populations worldwide. The prevalence of herds with current or recent infections can be over 70 percent (Beaudeau 2001, Houe 1999). BVDV infection is often subclinical or causes mild and non-specific clinical signs. However, transplacental infection can lead to reproductive disorders, teratogenic defects or birth of immuno-tolerant, persistently infected (PI) calves (Baker 1995). PI animals are considered the most important source of infection, excreting high amounts of virus continuously during their life via nasal discharge, saliva, semen, urine, faeces, tears and milk (Fray, et al. 2000). PI animals often survive for less than 24 months due to compromised immune competence and subsequent infections (Houe 1995). However, some female PI animals may survive beyond 24 months and give birth to PI offspring before being culled or succumbing to mucosal disease. In contrast to the high prevalence of BVDV in cattle populations, PI animals are only a small percentage (1-2%) of the population (Littlejohns and Horner 1990). BVDV infection is present in New Zealand with an estimated population prevalence of about 60% and prevalences of PI animals in the range of 1-5% (Horner 1996, Littlejohns and Horner 1990). However, there is no information on the herd-level prevalence of herds with one or more PI animals. No industry level control programmes have been put in place against this disease in New Zealand and vaccination guidelines are provided by vaccine manufacturers only. Thus, BVDV control is entirely left to the discretion of the individual dairy farmer supported by veterinary advice from practitioners and pharmaceutical companies.

There are several tests available to determine infection status of a dairy herd (Sandvik 1999, Valle 2000). Tests either detect BVD virus or antibodies to BVDV. They use either blood or serum from several animals per herd or bulk tank milk. Mainly ELISA based antibody detection tests are currently being used either on bulk tank milk (Niskanen et al. 1991, Bitsch 1997), or on serum from young stock or milk from first calving cows (Houe 1992, Houe 1994, Lindberg 1996). The main aim of herd-level testing is to identify herds with current infection for testing and removal of PI animals. In Sweden, repeated bulk tank milk testing is used to detect a rise of antibody levels indicating herd infection status (Alenius et al 1997). In Norway, bulk tank milk testing

in combination with testing milk from first calving cows and serum from young stock are used for the same purpose (Valle 2000).

The main objective of this study was to evaluate the test accuracy of a BVD serum antibody ELISA test applied to bulk tank milk samples to differentiate currently infected from non-infected herds. A high proportion of BVD antibody positive calves aged 6 – 18 months was regarded as an indicator for the presence of at least one PI calf suggesting active herd infection (Houe 1994). The other objective was to use the optimal cut-off value of the bulk tank milk ELISA test for an estimate of the herd-level prevalence of BVDV infections among dairy herds of the North Island of New Zealand.

Materials and Methods

Data collection

Bulk tank milk (BTM) samples

A simple random sample of 800 herds was targeted from a population of dairy herds in the North Island of New Zealand. The sample size was estimated to provide 80% power to detect a prevalence of 50% BVDV infection with 95% confidence and a design effect of 2 to adjust for within herd correlation.

Sampling was carried out by the Animal Health Laboratory of the Livestock Improvement Corporation limited (LIC), through liaison with the Fonterra Co-operative Group. The samples were obtained from the catchment area of the Saitl Laboratory (Hamilton) including the regions of Waikato, Bay of Plenty and Northland. These regions comprise 49% (n = 6705) of total dairy farms in New Zealand (Dairy Statistics, 2000 - 2001).

Herds were randomly selected at the Saitl laboratory during the week of 4th March and 11 March 2002. Thirty ml of whole bulk tank milk were obtained from each selected herd. The milk samples were then chilled and sent to a certified commercial laboratory¹ for BVD antibody testing. Each test tube was bar coded with a dairy company number

¹ Alpha Scientific, 57 Sunshine Avenue, Hamilton, New Zealand

and a supplier number. The supplier numbers were matched to the Livestock Improvement National database to source herd codes, addresses and other information. Additional information on herd management practices relevant to BVDV infection was acquired through a questionnaire sent to all sampled herds by mail.

Blood samples

Raw values of bulk tank milk antibody tests as determined by ELISA, were divided into quartiles of inhibition percentage (%SP). Random samples of 20 herds were selected from each of the quartiles. The sample size of 80 herds was based on budget limitations. Farmers responsible for selected herds were then contacted for inclusion in the study. Farmers were included in the study if they agreed to participate and if their young stock had no contact with cattle from other farms. The veterinary practices servicing these farms were then contracted for blood sampling. Practitioners were requested to take blood samples from 15 young calves aged 6 to 18 months and 15 milking cows. A systematic random sampling procedure was provided to each veterinary practice. The samples were sent to the same commercial laboratory that tested the BTM samples.

Milk and blood Testing

An indirect blocking ELISA test (Pourquier BVD/MD p80 milk ELISA test, Institut Pourquier, Montpellier, France) was used to test for bulk milk BVDV antibodies. All microtitre plates were coated with the protein p80 by a specific monoclonal antibody WB 112. Milk samples were diluted 1:8 and dispensed into the reference and test wells. After incubating the plates for 90 minutes at 37°C, the plates were washed and a monoclonal antibody to anti-bovine IgG conjugated with horseradish peroxidase was added. After 30 minutes incubation at 37°C, the plates were washed again and an enzyme substrate was added to the plates forming a blue compound and becoming yellow after blocking. The intensity of the colour proportional to the level of BVDV specific antibodies in the milk and was measured by the corresponding optical density. The optical density (OD) was read at 450 nm and reference wavelength of 630nm. Corrected OD values were calculated by subtracting from each OD reading the corresponding blank OD value (read from the well in which antigen had not been added). The percentage inhibition (%SP) was expressed by the formula:

$$\%SP = (\text{corrected OD}_{\text{sample}} / \text{corrected OD}_{\text{positive control}}) \times 100\%.$$

The sero-prevalence within herds was classified according to Beaudeau (2001): %SP of bulk tank milk lower than 20% was considered to be from herds with a within herd seroprevalence lower than 10%, %SP between 20% and 40% from herds with within herd seroprevalence between 10% and 30%, and %SP greater than 40% from herds with a within herd seroprevalence higher than 30%.

Blood samples were tested by the SERELISA™ BVD test, a p80 antibody mono blocking ELISA test. Ten-fold dilutions of serum samples were placed in wells sensitised with the BVD p80 protein and incubated in ELISA plates for 14 to 18 hours at 5°C. The microplates were washed four times with PBS-Tween, then 100 µl of horse radish peroxidase conjugated anti-bovine antibody (diluted 1:20 in conjugate diluent) were added and incubated at 5°C for 1 hour. After four washes, substrate was added and plates were incubated for 30 min at room temperature. Optical density (OD) was read with an ELISA reader at a wavelength of 450 nm and reference wavelength of 630 nm. Test results were validated and expressed as positive or negative for each animal.

Data processing and analysis

To view the source of herds in the sample, geographical point locations were plotted into a digital map of the North Island of New Zealand. All data were stored in a relational database (Microsoft Access version 7.0, Microsoft, Redmond USA).

The number of BVDV sero-positive young stock and cows were compared with %SP of bulk tank milk. Graphical methods were used for the comparisons. Means of the continuous variable (%SP) were compared using an independent t-test. To differentiate between herds with current infection (positive) and herds without current infection (negative), a cut off for the number of young stock testing positive to BVDV antibodies was selected. The test performance of the bulk tank milk ELISA at various levels of %SP was evaluated at this cut off.

Logistic regression was used for the evaluation. The dependent variable was a binary variable expressing the herd status based on the cut-off for the number of young stock testing positive to BVDV antibodies. Explanatory variables in the model were bulk tank milk S/P%, herd size, average milk solids per cow per year, BVDV vaccination status (yes/no) and heifer rearing management. The practice of heifer rearing was based on

questionnaire responses and categorised as 'heifers homebred and home-reared', 'heifers home-bred and reared on a different farm', and 'some heifers purchased from shows/fairs'. These categories were mutually exclusive. Variable effects were tested for significance by the likelihood ratio test (LRT), and model building done by stepwise removal of non-significant variables ($p > 0.10$) from the full model including all variables. The output of the logistic regression model was used to compute the sensitivity and specificity of the bulk tank ELISA test. Sensitivity was defined as the probability that a positive herd (4-15 sero-positive calves) had a %SP larger than a specific cut-off for %SP. Specificity was defined as the probability that a negative herd (0-3 sero-positive calves) had a %SP lower than or equal to that %SP cut-off. Thus, sensitivity and specificity were a function of the cut-off values chosen for %SP to discriminate positive herds from negative herds.

The optimum cut-off value for %SP was determined by means of the receiver operating curve (ROC), a plot of sensitivity (y-axis) by $1 - \text{specificity}$ (x-axis).

Based on the optimal cut-off, %SP of the entire sample of bulk tank milk were used to compute regional point estimates and confidence intervals of infected herds.

Results

Bulk tank milk BVDV ELISA antibodies

A total of 724 herds were sampled for BVDV bulk tank milk antibody testing. Based on the supplier number of these herds, 668 herds could be associated with region of origin. No supplier numbers or other herd identification was available for the remaining 56 herds. Figure 1 shows the geographical location of the tested herds. The average number of cows per herd was 248 (range 23-1340) with an average annual production of 315 kg milk solids per cow (range 160 - 488).

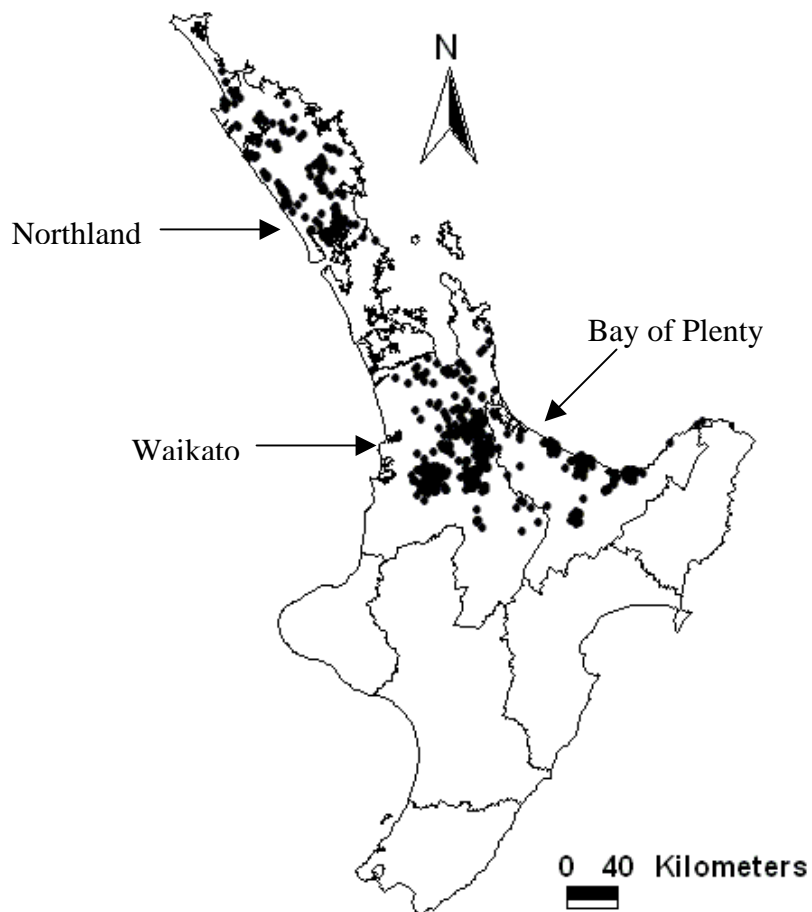


Figure 1. Geographical location of herds from the North Island of New Zealand, which were tested for BVDV antibodies in bulk tank milk.

Using the classification of Beaudeau (2001), 3 herds (0.4%) were classified as having a less than 10% within herd prevalence, 70 herds (9.7%) had 10 to 30%, and 651 herds (89.9%) had greater than 30% within herd prevalence (Table 1). There was no regional

association with herd prevalence (chi square = 7.4; $p > 0.1$) indicating that BVDV infections across the three regions were similar.

Table 1. Regional classification (Beaudeau 2001)* and sampling fraction of 724-bulk tank milk samples, by BVDV within herd prevalence according to the ELISA test classification (CI = confidence interval in %).

Region	Within herd prevalence			Total herds In sample	Total herds in region	Sampling fraction % (95% CI)
	<10%	10-30%	>30%			
Bay of Plenty	8 (6.2)	121 (93.8)	129	782	16.5 (14.1-19.2)	
Northland	12 (7.1)	157 (92.9)	169	1455	11.6 (10.0-13.3)	
Waikato	1 (0.3)	45 (12.2)	324 (87.6)	370	4468	8.9 (8.3-9.6)
Unclassified	2 (3.6)	5 (8.9)	49 (87.5)	56		
Total (%)	3 (0.4)	70 (9.7)	651 (89.9)	724	6705	10.8 (10.0-11.5)

%SP lower than 20% = <10% within herd prevalence

%SP between 20 and 40% = 10 - 30% within herd prevalence

%SP greater than 40% = >30% within herd prevalence

The average inhibition percentage (%SP) for the entire population was 62.4% (CI 61.2 – 63.7%). Figure 2 shows the distribution of bulk tank milk %SP for all herds. The Spearman correlation between %SP and herd size was 0.083 and the correlation between %SP and lactation average in milk solids per cow was –0.043. These correlations were extremely low and non-significant indicating that herd size and production level were not associated with the level of BVDV antibodies in bulk milk.

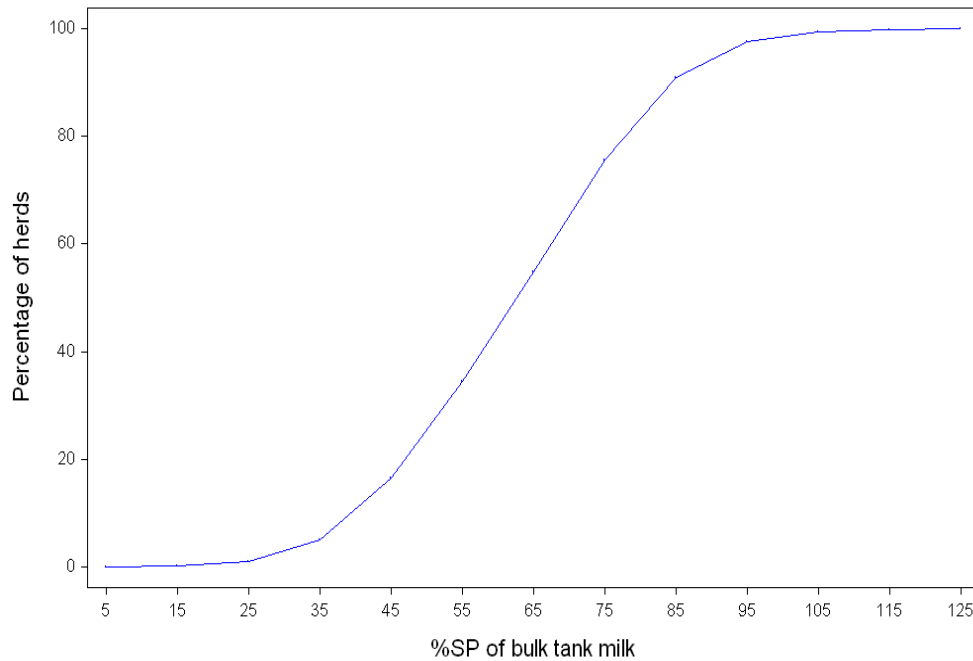


Figure 2. Distribution of %SP of bulk tank milk for 724 herds tested for BVDV antibodies.

Blood test results

Blood samples from 741 calves and 645 cows obtained from 61 herds were available for analysis. Calf-samples were from 50 farms; 32 of these farms also submitted cow-samples. One farm submitted only 6 calf-samples but complete sets of 15 calf-samples were available from the other 49 farms. Cow-samples were obtained from 11 farms that had not submitted calf-samples and these farms were not included in the analysis. Thus, cow-samples were available from a total of 43 farms. Test results are summarised in Table 2.

Table 2. Comparison of herds classified as non-infected (0- 3 antibody positive calves, 6 – 17 months old) and herds classified as infected (> 3 antibody positive calves, 6 – 17 months old).

	Non-infected herds	Infected herds
<u>Calves:</u>		
Number of herds	34	16
Number of calves tested	501	240
Number of BVDV AB positive calves	27	183
Percentage of AB positive calves	5.4	76.3
95% Confidence interval	3.4 to 7.4	70.9 to 81.7
Mean %SP of bulk tank milk	63.3	84.3
Standard error	3.1	5.2
95% Confidence interval	57.0 to 69.5	73.1 to 95.4
<u>Cows:</u>		
Number of herds	28	4
Number of cows tested	420	60
Number of BVDV AB positive cows	186	26
Percentage of AB positive cows	44.3	43.3
95% Confidence interval	34.9 to 53.7	18.5 to 68.1
Mean %SP of bulk tank milk	58.6	58.8
Standard error	3.0	15.6
95% Confidence interval	52.5 to 64.7	43.2 to 74.4

The average age of young stock at sampling was 11 months with a range of 6 – 17 months. The age of calves was 4 months higher on average in the group of herds with 4-15 sero-positive calves classified as having active BVD infection. Initial descriptive analysis indicated that most blood samples submitted were from herds with low %SP of BTM. Veterinary practices were then contracted for blood sampling herds with high %SP, as a result calves from herds with high %SP were sampled at a later stage of the study.

Figure 3 shows the frequency of herds by the number of calves testing positive for BVDV antibodies in a sample of fifteen. In Table 2 the 50 herds were divided in two groups, a 'non-infected' group with 3 or less antibody positives calves and an 'infected' group with 5 or more positive calves. This resulted in 34 non-infected herds with 27 sero-positive of 501 calves tested (5.4%). Among these 34 non-infected herds, 16 herds had no sero-positive calf among 15 tested. The 16 herds regarded as infected had 183 antibody-positive calves among 240 tested (76.3%). The cows of 5 herds in each group had been vaccinated against BVDV in 2001. There was no association between vaccination against BVDV and BVD infection status of a herd.(Chi square = 1.86, df=1). The proportions of BVDV antibody positive cows were similar in infected and non-infected herds (43.3% and 44.3% respectively; Table 2).

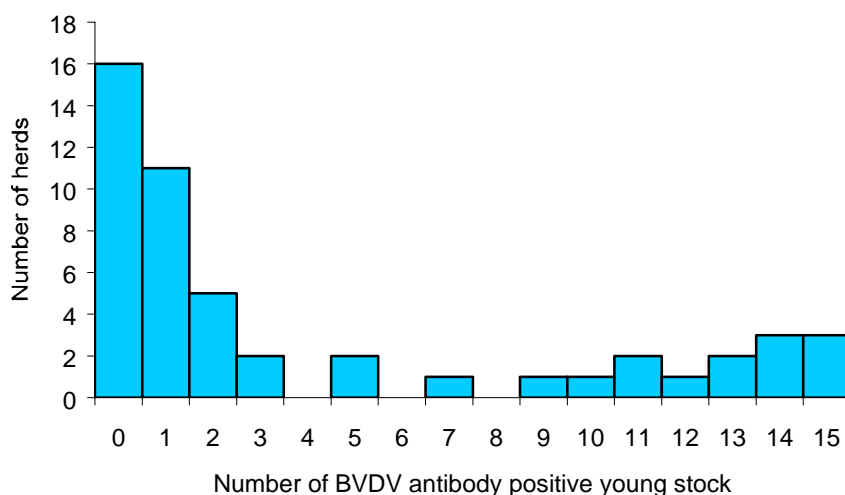


Figure 3. Frequency distribution of number of BVDV antibody carriers among samples of 15 young stock taken from each of 50 herds.

Sensitivity and specificity of the bulk tank milk ELISA test

Figure 4a shows a scatter plot of %SP versus number of BVDV antibody positive calves. Given that 3 or less antibody positive calves was the threshold for non-infected herds, the plot suggested a cut-off for %SP at 80%. This resulted in 3 of 34 non-infected herds being misclassified as infected (i.e. %SP > 80%) and 3 of the 16 infected herds misclassified as being non-infected.

Figure 4b shows that the number of antibody positive cows correlated positively with %SP of bulk tank milk. However, only 4 herds of which cow samples were available were infected based on their calf-sample. One of these had a %SP greater than 80%. All other herds were below 80%SP.

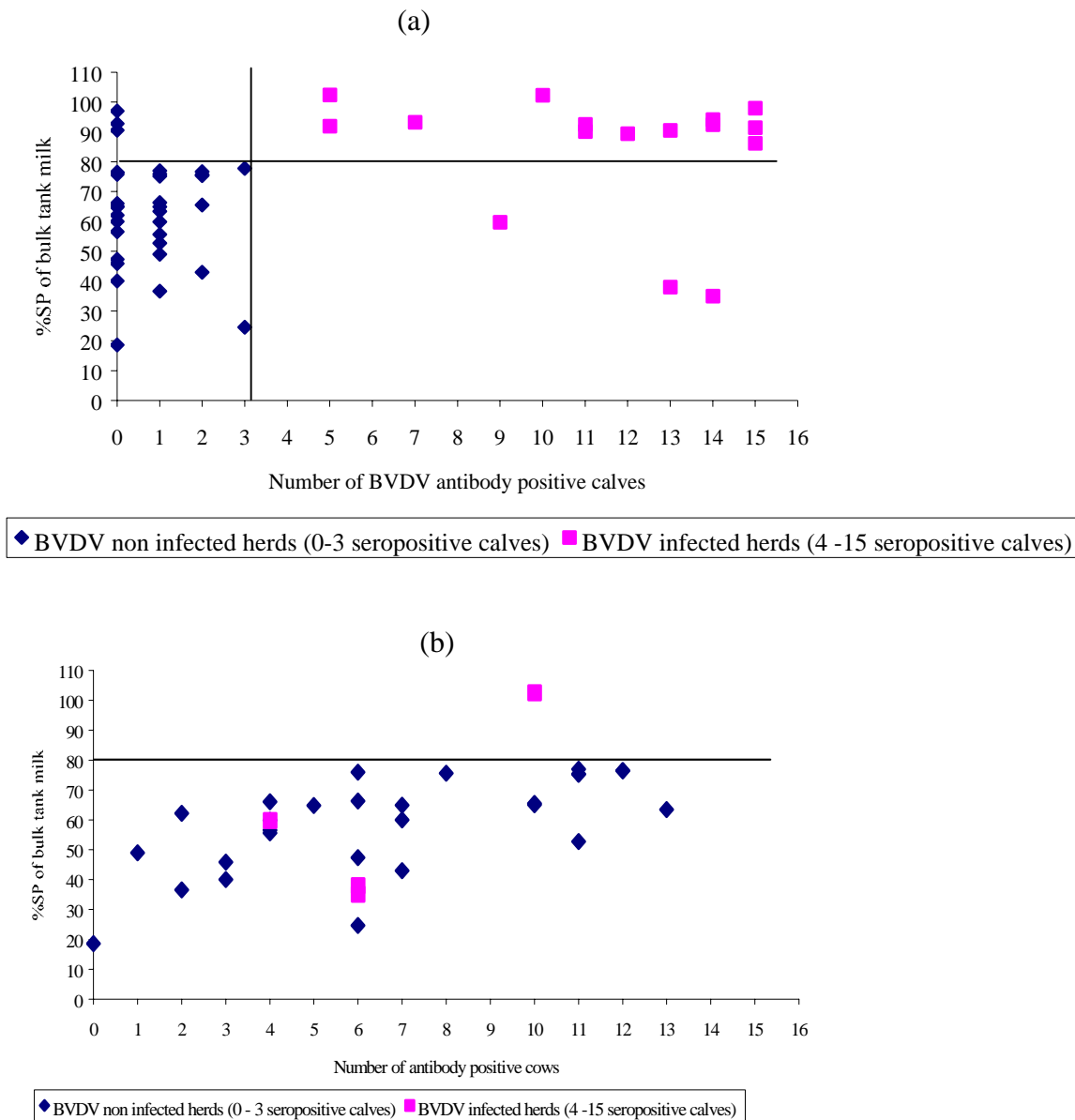


Figure 4. Scatter plot of %SP based on optical density of bulk tank milk, plotted against (a) number of BVDV antibody positive calves (6-17 months), (b) number of BVDV antibody positive cows

The result of the logistic regression model indicated that only %SP of bulk tank milk was significantly associated with the probability of a herd being infected. Other

variables were not significant. For every 10% increase in %SP of bulk tank milk, the probability of infection in a herd increased by 7% (Table 3).

Table 3. Logistic regression results of variables included in the model for herd BVDV infection status (50 herds in the model, residual degrees of freedom = 42).

Variables in the model	B	S.E.	Wald	df	Sig.
Constant	-0.049	4.265	0.000	1	0.991
%SP	0.067	0.024	7.784	1	0.005
Herd size	-0.003	0.003	0.523	1	0.469
Milk solids (kg) per cow	-0.015	0.012	1.624	1	0.203
Replacement heifers homebred and home reared	0.725	0.908	0.639	1	0.424
Replacement heifers homebred and reared in separate mobs on a different farm	-0.108	0.853	0.016	1	0.899
Animal are purchased from shows/fairs	-0.130	0.950	0.019	1	0.892
Vaccinated against BVDV	-0.891	0.965	0.853	1	0.356

The receiver operator curve (Figure 5) confirmed that 80% was the optimum cut-off value for %SP as already suggested by Figure 4a. This resulted in 81.2% sensitivity and 91.2% specificity.

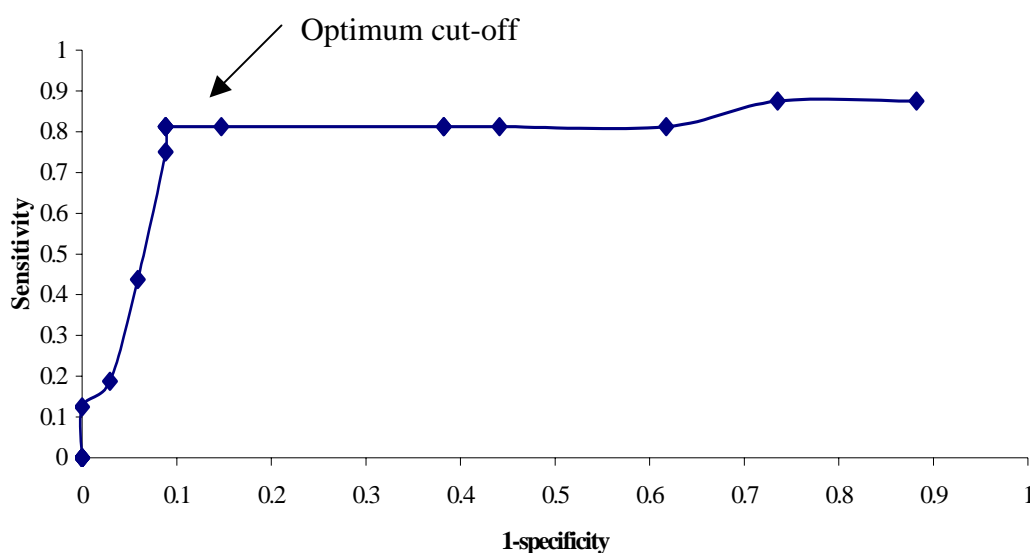


Figure 5. ROC curve for %SP decision thresholds of the ELISA test applied to bulk tank milk to identify BVDV infected herds.

Table 4 shows the regional distribution of herds with and without persistently infected animals using 80%SP of bulk tank milk as the cut-off discriminating between infected and non-infected herds. There were no significant differences in the herd prevalences between regions (chi square = 5.49, df = 3)

Table 4. Regional distribution of infected herds

Region	N	Infected Herds (%)	95% confidence interval (%)
Bay of Plenty	129	8.5	3.7 -13.3
Northland	169	14.	9.4 - 20.1
Waikato	370	15.9	12.2 - 19.6
Unclassified	56	19.6	9.2 - 30.0
Total	724	14.6	12.0 - 17.1

Discussion

As figure 1 illustrates, the herds used in this study were randomly distributed over the 3 regions. With an overall sampling fraction of 10.8% and the fact that herd size and production were similar to the population average, the study herds can be regarded as a random subset of the population of dairy herds in the 3 regions.

Initially, 80 herds were randomly selected from each of the 25-percentiles of %SP for testing BVDV antibodies in calves and cows. Because some farmers grazed their calves on separate farms and due to a limited study budget, complete sets of samples from both calves and cows could not be obtained from all herds.

Herds with a current BVDV infection are expected to have high levels of antibodies to BVDV in bulk milk (Niskanen et al. 1991, Bitsch et al 1995, Drew et al 1999). This also applies to herds that have recently become free from active infection due to the presence of long lasting antibodies. The basis for testing young stock is the high prevalence of sero-positive calves when one or more PIs are present. This is caused by the rapid spread of virus from PIs to in-contact stock (Houe 1995). In general all animals born after the removal of the last PI will presumably be seronegative except for calves born

with antibodies resulting from an in utero infection that occurred in late gestation shortly before the last PI animal has been removed. As a result, detection of antibody-positive individuals in a spot test directed towards calves is an indication of active infection. If antibodies are not present in a sample of calves, it is inferred that the herd has not been infected during the lifetime of the animals (Houe 1991;1995, Drew 1999, Brownlie 1990, Lindberg 2001) and that no new infection occurred during the last pregnancy of any dam. Due to a distinct seasonal calving pattern in most New Zealand dairy herds, a negative spot test among 6 to 18 month old calves suggests that the herd has not had contact with PI animals for the previous one and a half years. In our study herds, 16 of 34 herds classified as being non-infected did not include a single antibody positive calf.

However, there were 18 calf mobs with 1-3 antibody carriers in herds regarded as non-infected. The cut-off of three was primarily based on the distribution of reactors (Figure 3) and on a similar study by Houe (1994). Possible reasons for calves testing positive in non-infected herds include contact to neighbouring herds by calves jumping the fence or calves born with antibodies resulting from an in-utero infection in late gestation shortly before the last PI animal was culled. Another reason is test misclassification due to lack of specificity (false positives).

Although some calf mobs from herds with a high %SP were sampled and tested later than mobs from low %SP herds, it is highly unlikely that this affected the seroprevalence of the calves. In the presence of PI animals, BVDV is transmitted within days (Littlejohns 1985; McGowan et al. 1993). In our study however, the shortest period between birth and testing was 6 months. The cut-off in this study was the same as the one reported by Houe (1994). Houe (1994), tested 42 Danish dairy herds for BVDV antibodies in bulk tank milk, and tested blood samples from 10 calves of each herd aged between 8 and 18 months. The herds of that study were classified as non-infected when they had 3 or less antibody carriers (24 herds) and as infected when 8 or more calves were antibody positive. Bulk milk %SP was generally higher in herds classified as infected.

The estimated prevalence of herds with active BVDV infection was 14.6% (Table 4) in the regions Northland, Waikato and Bay of Plenty. This is an estimate of the prevalence

of herds with PI calves while Beaudeau estimated herds with a high prevalence of sero-positive cows. Few studies have investigated the prevalence of herds with PI animals. A survey of all cattle in 19 Danish dairy herds with unknown status of BVDV infection showed that 10 herds (53%) had PI animals in varying numbers (Houe and Meyling 1991) while 9 herds had no PI animals. Another survey of 20 herds in the USA showed that 15% of the herds had PI animals (Houe 1996). Vaccination had been carried out in 15 of the 20 American herds but in none of the Danish herds. In a study carried out in Germany, 45% of 329 herds had PI animals (Frey et al. 1996). Thus, the prevalence in our study areas was comparatively low.

According to the bulk tank %SP classification of Beaudeau (2001), 90% of all herds in the study area would have a high within herd prevalence of BVDV antibody positive cows (Table 1). The author based this relationship on milk samples from 135 herds, and this was quantified by using a general linear model and receiver operating characteristic analysis to define classes of percentage inhibition in the bulk milk associated with within-herd prevalence. However, the French dairy production system is entirely different to that of New Zealand. The distinct seasonal calving pattern, short replacement periods, and keeping replacement stock in more homogeneous age groups, may reduce BVDV transmission rates in New Zealand dairy herds. On the other hand, larger herd sizes in New Zealand probably increases the rate of replacements from other herds, thus increasing the between herd contact rate. Therefore, Beaudeau's classification of bulk milk %SP for within herd prevalences might not be appropriate for New Zealand. Since cow blood samples were available from only 4 infected herds, the association between the proportion of sero-positive cows and %SP in our study was relatively weak and therefore could not be used to differentiate between infected and non-infected herds.

We conclude that bulk tank milk can be used to determine BVDV infection status of dairy herds in New Zealand. The test could be used for BVDV control at farm or at industry levels.

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