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The epidemiology of enzootic bovine leukosis in dairy cattle in New Zealand

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ABSTRACT

This thesis is a part of the research components associated with the National Enzootic Bovine Leukosis (EBL) Control Scheme in New Zealand. The objectives were to investigate the risk factors and temporal pattern of Bovine Leukaemia Virus (BLV) infection in dairy cattle. A case-control study was conducted using questionnaire information collected from 719 farms throughout New Zealand in 1996 and in 1997. A longitudinal study was conducted over the same period in 4 high BLV prevalence farms around Manawatu and Wairarapa regions. The results of the studies were:

(1) The presence of BLV infection was strongly related to management practices. Two predictors, namely large herd size and the purchase of stock from external sources, were strongly associated with the presence of BLV infection within herds. The number of years since farm establishment was also a risk factor which was most likely related to purchase patterns. Newly-established herds or large herds tend to buy stock from external sources and obtaining stock off-farm is likely to introduce infection. In addition, performing pregnancy testing late in the milking season and the absence of hygiene measures when undertaken mechanical dehorning of calves were significant risks which may explain within-herd transmission. The characteristics of managers from BLV-positive herds could be described as "progressive" since they were young, better educated and made used external advisers to assist with decision making.

(2) The presence of BLV infection can be diagnosed precisely by blood test using the ELISA technique. Vertical transmission may play an important role depending on the practice of calf rearing. New cases of BLV infection principally developed in animals within the first two years of age. Prevalence and cumulative incidence of disease are mainly influenced by the existing prevalence in the herd, rather than differences in management practices. The risk of seroconversion depends on herd management factors rather than factors relating to animals. Infected animals are likely to have higher production capacity but this may be due to the fact that these animals were retained in the herd because they were high producers. The factors that predict production capacity are BLV infection status, herd and interaction between herd and BLV status.

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As my period of postgraduate study comes to a close, I am reminded of the early days when I knew little of veterinary epidemiology, statistics and computing. These two years have made me achieve in what I have never expected and never realised in my potential.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 - Introduction

Enzootic Bovine Leukosis (EBL) is classified as a List B communicable disease under the OIE regulations. EBL has long been recognized as the most common neoplastic condition of dairy cattle and is caused by Bovine Leukaemia Virus (BLV) (Ferrer 1980). Evermann (1992a) has summarised the features of BLV infection in cattle as follows:

1. BLV is species specific.
2. BLV replicates via reverse transcriptase and can persist for indefinite periods within cells in an integrated state within the host DNA.
3. BLV does not carry its own transforming gene like other transforming oncoviruses. Instead it interacts with cellular genes, resulting in neoplasia.
4. Most cattle older than six months, once they become infected with BLV, are infected with the virus for life.
5. *Once an animal is infected with BLV the onset of EBL is unpredictable.*
6. A small percentage (1 to 5%) of BLV-infected cattle develop EBL.
7. There are currently no vaccines available to prevent BLV infection or EBL.

BLV is highly associated with cells of the lymphoreticular system. The virus should be regarded as infectious but not highly contagious.

Enzootic Bovine Leukosis is known by several terms including leukemia, leukosis, lymphosarcoma, and malignant lymphoma. EBL is characterized by proliferation of neoplastic lymphocytes to form either discrete solid tumour masses and/or a neoplastic infiltration of various tissues and organs.

BLV infection of cattle has become a concern to the New Zealand cattle industry for three primary reasons. Firstly, BLV infection is associated with EBL and as a result is a potential source of involuntary loss to cattle producers. Secondly, because some countries have successfully eradicated BLV infection the presence of BLV infection within the national herd is a potential barrier to international trade. Thirdly, the relationship between BLV infection and human health is unknown. Given that the prevalence of BLV infection in New Zealand dairy cattle is low (Hilbink 1991); (Burton *et al.,* 1997) and acknowledging the importance of these three areas of

concern it is timely that the New Zealand dairy industry embarks upon a programme of voluntary eradication of the infection from the national herd.

This thesis contains the results of studies conducted to address two important epidemiological issues related to the eradication of BLV from the New Zealand dairy industry, that is:

1. a case control study to identify farm level characteristics and practices that are associated with the presence of BLV infection, and
2. a longitudinal study of cattle from BLV-positive herds aimed at identifying management and temporal risk factors for BLV seroconversion.

A knowledge of these risks will help in defining effective control programmes for BLV-positive herds and hasten the eradication of the virus from the national herd.

Throughout this thesis the acronym EBL has been used to describe the syndrome of Enzootic Bovine Leukosis, that is clinical lymphosarcoma. The acronym BLV has been used for Bovine Leukaemia Virus, the aetiological viral agent that causes Enzootic Bovine Leukosis.

CHAPTER 2 - Literature review

General information

Aetiology

The Bovine Leukaemia Virus (Figure 2-1) is an oncogenic RNA virus belonging to the Retroviridae family. Though some studies have suggested that T lymphocytes and monocytes are susceptible to infection it is currently believed that the B lymphocytes are the only mononuclear cells in peripheral blood that are infected with BLV to any great extent (Mirsky *et al.*, 1996).

Like other retrovirus species, the BLV genome is made up of one molecule of single-stranded RNA with a molecular weight of 7 - 10 x 10^6 dalton. This virion has a diameter of 60 - 150 nm depending on the viral cultivation technique employed. The centrally located nucleoid, with a diameter of 40 - 90 nm, is surrounded by an intermediate membrane. The core itself consists of a subunit of hexagonal shape and the outer envelope shows "knob-like" surface projections of about 8 nm (Straub 1981) (Figure 2-2).

BLV matures at the cell surface by a characteristic budding process (Ferrer 1980) but its propagation is dissimilar to other viral diseases of cattle in that secretions from sick animals do not contain large quantities of the virus (Straub 1981).

The viral proteins that determine the immunological response in the host are divided into two major groups: the non-glycosylated structural proteins and the glycoproteins. These are named according to the composition and their molecular weights. The non-glycosylated proteins have molecular weights between 10000 and 24000 daltons whereas the glycoproteins (gp) (most likely contained in the knob-like structures on the outside surface of the viral envelope) have molecular weights between 45000 and 70000 daltons. Only two of these major groups have played an important role for serological diagnosis, namely the non-glycosylated peptide with a molecular weight 24000 (p24) and the glycoprotein peptide with a molecular weight of 61000 (gp51) (Burny *et al.*, 1978), (Johnson *et al.*, 1992).

This BLV virus shows no antigenic cross reaction with other common bovine viruses or other oncorna viruses (Ferrer 1980) thus having unique antigenicity.

Although minor differences between various isolates have been found, immunologic tests have not identified more than one strain of the agent (Miller 1986). Miller (1986) has also identified that the virus undergoes periodic antigenic change.

Pathogenesis

The time required to develop clinical EBL, if it occurs, may last from several months to several years and differs between individuals. The virus is present mostly in lymphocytes and can be found in blood, milk and tumour masses. Transmission of BLV may be horizontal or vertical with the horizontal route being the more important of the two (Ferrer 1980), (Johnson *et al.*, 1992), (Radostits *et al.*, 1994). Horizontal transmission requires the transfer of infected lymphocytes from one animal to another. The most common methods of transmission under natural conditions include gouge dehorning with a common instrument and procedures that permit the transfer of blood from one animal to another (Hopkins *et al.*, 1997).

The rate of vertical transmission ranges from 0.5 to 18% (Hopkins *et al.*, 1997). The most common form of vertical transmission of the virus is via the ingestion of colostrum and milk. The rate of transmission by this route is inconclusive due to the small sample sizes that have been used in most studies.

Cattle infected with BLV do not produce significant amounts of cell-free virus in their tissues, secretions, or excretions. Since the virus is highly cell-associated, viraemia does not occur after transmission like most viral infections (Jubb *et al.*, 1985). The mode of replication in infected cells is characteristic. Viral RNA is copied with the aid of a specific viral enzyme into a "mirror image" DNA molecule. This DNA (known as the provirus) is integrated into the host's chromosomal DNA and becomes a permanent part of the infected cell's genetic information, even in the absence of further virus replication and in the presence of viral specific antibody. After the host is infected, the virus survives in a proviral state in bone marrow-derived lymphocytes, perpetuating and multiplying coincidentally at each subsequent cell division. Once infection has been established, antibodies are produced and persist for life (Jubb *et al.*, 1985), (Miller 1986).

Whether or not an animal becomes persistently infected with BLV or develops an immunological response depends principally on its genetic constitution (Ferrer 1980) and/or the infective dose of virus received (Roberts *et al.*, 1985b). Bovine

Lymphocyte Antigens (BoLA) are thought to be responsible for differing animal immune responses, with different BoLA types reported for animals of different breed and age (Palmer *et al.,* 1987). The mechanism controlling the maintenance of latency, lymphoproliferation in the persistent lymphocytosis phase (if it occurs) and initiation of tumourgenesis are unknown. A summary of the pathogenesis of BLV infection is shown in Figure 2-3.

The possible outcomes of BLV infection are outlined in Figure 2-4 and are described in detail as follows:

Failure to become persistently infected

In certain circumstances it has been shown that animals can be exposed to BLV yet show no subsequent signs of the virus either clinically or serologically. The probability of this outcome occurring depends on: (1) the host's defence mechanisms, (2) the route of infection, and (3) the dose of virus (Evermann 1992a).

Development of an immune response

The development of a detectable immune response is the most common sequelae to BLV infection and the risk of infection establishing a response in the host increases with the number of lymphocytes present in the infecting blood (Hopkins *et al.,* 1997).

Numerous studies have investigated the size of infective blood dose required to achieve an immune response. Van der Maaten (1978) reported that a minimum of 2500 lymphocytes from a BLV seropositive animal (approximately 0.5 μL of whole blood) inoculated intradermally could induce infection (DiGiacomo 1992c). A dose of 1 μL (approximately 4500 lymphocytes) of whole blood from BLV seropositive cows induced infection in calves when administered intramuscularly, intravenously, subcutaneously or intradermally (Evermann *et al.,* 1986b). Evermann *et al.* (1986) demonstrated that the greater the number of lymphocytes innoculated, the shorter the time taken to seroconvert. Cows inoculated with 1μL of infected blood seroconverted to BLV within 14 weeks whereas those inoculated with 10 μL seroconverted within 8 weeks.

It has been hypothesised that the risk of BLV transmission is higher in herds where there is a high prevalence of BLV (Hopkins *et al.,* 1997). Since persistent

lymphocytosis appears in about one-third of BLV-infected animals there will be more animals manifesting PL in high prevalence herds than in low prevalence herds. Contact with cattle that have PL increase the potential for spread of infection (DiGiacomo 1992c). Several studies have demonstrated that lymphocytes obtained from BLV-infected cattle with persistent lymphocytosis were infective at lower doses compared to lymphocytes from BLV-infected cattle with normal lymphocyte counts (Buxton *et al.,* 1984), (Burny *et al.,* 1988) (DiGiacomo 1992c).

Following exposure the virus migrates to the spleen by day 8 and by day 14 the virus may be found in the host's circulating lymphocytes. Infection with BLV and the production of antibodies to BLV is permanent. This is due to the fact that the virus locates itself in lymphocytes in a covert, non-productive state resulting in an inability of developed antibodies to arrest and remove the infection. Furthermore, the virus is capable of undergoing periodic antigenic change thus circumventing a curative immune response (Radostits *et al.,* 1994). Cattle develop antibodies to BLV and remain carriers of the virus for life, potentially transmitting the virus to others within the herd (Radostits *et al.,* 1994).

Development of persistent lymphocytosis

Classical studies of BLV infection describe the development of a persistent lymphocytosis as a preclinical or subclinical stage of Enzootic Bovine Leukosis. Ferrer (1980) disagreed with this, estimating that less than 10% of BLV-infected animals with persistent lymphocytosis go on to develop malignancy. The presence of unintegrated proviral BLV DNA in those animals with a persistent lymphocytosis is thought to be a potential marker for identifying those cases which will go on to develop neoplasia (Reyes *et al.,* 1996). Reyes and Cockrell (1996) demonstrated that unintegrated proviral BLV DNA part was detected in 100% of cattle that were seropositive to BLV with PL (10 of 10), 53% of BLV-seropositive cattle that were haematologically normal (18 of 34), and in 0% of BLV-seropositive cattle that developed Enzootic Bovine Leukosis (0 from 8). Conversely, a Canadian study found proviral BLV DNA in five out of seventeen cattle with Enzootic Bovine Leukosis (Jacobs *et al.,* 1992).

Development of Enzootic Bovine Leukosis

Though it is believed that genetic factors play an important role in determining which BLV-positive animals go on to develop EBL, the susceptibility to development of lymphosarcoma is an inherently difficult problem to study because of the low incidence of tumours in BLV-infected cattle (Lewin *et al.,* 1986). Recently, there has been speculation about the effect that other viruses may have in increasing or decreasing the pathogenicity of the BLV virus. Those viruses thought to be involved with the pathogenesis of BLV are the other bovine retroviruses (bovine immunodeficiency-like virus-BIV) and the bovine herpes viruses (Cockerell *et al.,* 1992), (Coats 1995).

Clinical signs

In the veterinary literature, three distinct clinical forms of BLV infection have been described (Radostits *et al.,* 1994). The fourth condition listed is not associated with BLV infection, but is associated with a clinical syndrome similar to EBL and as a result, a description is included for clarity.

1. Bovine Leukaemia Virus infection
2. Bovine Leukaemia Virus infection with persistent lymphocytosis
3. Enzootic Bovine Leukosis
4. Sporadic Bovine Leukosis

BLV infection

In this form of BLV infection there is no sign of illness or production loss (Ferrer 1980), (Radostits *et al.,* 1994). In this state animals are potential shedders of the virus via infected blood and/or in utero transmission of cell-free virus that crosses the placenta. BLV-positive animals have the potential to develop EBL at any stage throughout their lifetime (Evermann *et al.,* 1992b).

BLV infection with persistent lymphocytosis

Approximately 30 - 33% of animals infected with BLV have been estimated to have a persistent lymphocytosis (that is, a total circulating lymphocyte count of greater than 7.5 x 10^9 cells/L) (Ferrer 1980).

Persistent lymphocytosis may be accompanied by subacute to chronic loss of condition and appetite, anaemia and muscular weakness which may be a precursor to the development of full-blown EBL (Radostits *et al.,* 1994). Ag this stage, cattle will have no neoplasia detectable (Johnson *et al.,* 1991a).

Enzootic Bovine Leukosis

This form of the disease is widely recognised as the classical manifestation of BLV infection (Ferrer 1980) though it should be remembered that only 0.1 - 5.0% of BLV infected animals actually develop the condition (Radostits *et al.,* 1994). EBL is said to be a maturity-onset disease (Jubb *et al.,* 1985) with the majority of cases occurring between the fourth and eighth year of life (Radostits *et al.,* 1994). In most cases clinical signs are vague with anorexia, weight loss and agalactia coinciding with the appearance of tumour masses. Animals will manifest a variety of clinical signs depending on the site of neoplasia and the resulting malfunction in the affected organs. For example posterior paresis or paralysis may occur when the spinal cord or peripheral nerves are involved. The rapid rate of tumour growth and high degree of metastasis result in rapid fatality (Ferrer 1980).

In the generalised form of EBL enlargement of the superficial lymph nodes occurs in 75 - 90% of cases and is often an early clinical finding. This may be accompanied by small (1 cm diameter) subcutaneous lesions on the flanks and on the perineum (Radostits *et al.,* 1994). Although the enlargement of the superficial lymph nodes is common the internal nodes may also be enlarged in the absence of superficial involvement.

A summary of the clinical findings associated with Enzootic Bovine Leukosis is shown in Table 2-1.

Sporadic Bovine Leukosis

As its name suggests, Sporadic Bovine Leukosis occurs infrequently in cattle. Sporadic Bovine Leukosis has no epidemiological association with BLV infection or other infectious agents. Sporadic Bovine Leukosis is mainly restricted to animals under 3 years of age. There are three main manifestations of this condition (Radostits *et al.,* 1994): (1) a juvenile form, which occurs in calves less than 6 months old and causes sudden generalised lymph node enlargement, (2) a thymic

form, which tends to occur in yearlings less than 2 years old and is more common in beef than in dairy cattle, and (3) a cutaneous form, which is rare.

Diagnosis

There are two main aspects of BLV diagnosis, namely: (1) the detection of clinical disease (either persistent lymphocytosis as a result of BLV infection or the detection of EBL), and (2) the detection of (subclinical) BLV infection.

As international trade restrictions are usually related to identifying the presence of BLV infection, recent research has been undertaken to emphasise the sensitivity and specificity of tests designed to detect BLV infection. Improvement in the sensitivity and specificity of diagnostic tests also assists research into the epidemiology of BLV infection, providing a means by which the course of infection and the disease can be monitored.

In this review, areas of BLV diagnosis will focus on: (1) haematology, (2) histopathology, (3) antibody-based testing, and (4) nucleic acid-based testing techniques.

Haematology

Haematology has been used as a means of detecting persistent lymphocytosis and as a tentative means of diagnosing EBL. The criterion used for persistent lymphocytosis is an increase in the absolute lymphocyte count of at least 3 standard deviations above the normal mean count that lasts for at least 3 consecutive months (Ferrer *et al.,* 1979b). The total white blood cell count rises from a normal level of 6 x 10^9 cells/L to as high as 150 x 10^9 cells/L. The majority of cells involved in persistent lymphocytosis are normal lymphocytes but atypical and abnormal forms are also present. There is no evidence that cattle with persistent lymphocytosis harbour neoplastic or aneuploid cells (Ferrer 1980).

Recently, counts of circulating B-lymphocytes has been used as a means of diagnosing BLV infection (Lewin *et al.,* 1988). Twenty-nine percent of cows seropositive to BLV and haematologically normal were shown to have their B-lymphocyte count two standard deviations above the mean calculated for seronegative herdmates. Given the sensitivity, specificity, and convenience of

serological methods of testing, it is unlikely that this method of BLV diagnosis is likely to become popular.

Histopathology

A conclusive diagnosis of lymphosarcoma requires histopathologic examination of a section of affected tissue obtained from biopsy or autopsy. A fine needle aspirate of an enlarged lymph node may provide a rapid and inexpensive diagnosis and the presumptive diagnosis is usually accurate.

In EBL neoplastic masses are grossly indistinguishable from tumours that occur in the sporadic form. The differentiation can be made by using either an ELISA or PCR test together with the distinction of the affected age group (Klintevall 1993).

Antibody-based tests

Antibody-based testing is the most practical method for screening for BLV infection. The most common tests are the agar gel immunodiffusion (AGID) test, the radioimmunoassay (RIA) test and the enzyme-linked immunosorbent assay (ELISA) test (Evermann 1992a).

The AGID test was the first serological test to use the internal protein p24 of the virus to detect antibody (Miller *et al.*, 1972). This technique was important for identifying cases which were previously diagnosed solely by haematology or histopathology. More lately the glycoprotein antigen (gp51) which detects specific antiviral envelope serum antibodies has been found to be more sensitive than protein p24 (Onuma *et al.*, 1975). The sensitivity and specificity of AGID test using gp51 has been calculated to be 98.5% and 99.5%, respectively (Monke *et al.*, 1992) (Radostits *et al.*, 1994).

Reliable detection of BLV antibodies differs between experimental and natural infections. In experimental infections, antibody can be detected using AGID within 2 - 4 weeks of inocculation with BLV (Evermann *et al.*, 1986b). In natural infections antibody can be detected using AGID as long as 12 weeks after infection (Evermann *et al.*, 1997). As a general recommendation, AGID is not advised as the serological test of choice soon after exposure to the virus (Johnson *et al.*, 1991a), (Evermann 1992a).

Although the AGID test is less sensitive than other serological assays, it is still used routinely in many parts of the world. Its advantages are that it is inexpensive and easy to perform and yields results that are quick, clear and easily interpreted (Evermann 1992a). Furthermore, it can make use of spoiled sera that may have been collected under poor field conditions (Bex *et al.,* 1979). The AGID test is officially recognised as an acceptable test by the Office International des Epizooties and has been successfully used for the control and eradication of EBL in several countries (Brenner *et al.,* 1994), (Jacobsen *et al.,* 1985).

The RIA test was first developed using ^{125}I-labeled BLV p25 antigen in 1976. More lately, gp51 antigen has been used to improve the test's sensitivity. RIA is suitable for diagnosing BLV infection soon after exposure (Radostits *et al.,* 1994) proving to be the most sensitive of all the serological tests available. For routine use however, RIA offers no advantages over the AGID test since it requires sophisticated equipment and it is not well suited for mass-screening purposes (Nguyen *et al.,* 1993).

Since its first development in 1980 (Yolken 1980) the ELISA test has been widely used for the diagnosis of infectious agents. In 1984 a study comparing the sensitivity and specificity of ELISA and AGID tests for detecting BLV infection found no appreciable difference between the two methods (Mammerickx *et al.,* 1984). More recently, the ELISA test for BLV has been shown to be the more reliable of the two with a sensitivity and specificity of 98.5% and 99.9%, respectively (Johnson *et al.,* 1992).

The BLV ELISA test is able to be used on milk samples which favours its use in practice. The disadvantages of assaying milk for BLV antibodies are the large dilution factor, the interference by lactogenic proteins, and the instability of antibodies in stored milk as a result of bacterial growth (Evermann 1992a). The sensitivity of the BLV ELISA test used for milk samples is equal to that of serum samples although antibodies in milk are approximately 27 fold lower than that in serum (Johnson *et al.,* 1992) In a recent New Zealand study comparing milk and serum BLV ELISA tests the relative sensitivity was 96.7% and the relative specificity was 90.2% while the positive and negative predictive values were 37.9% and 99.8% respectively (Hayes *et al.,* 1997).

The BLV ELISA test has also been used for bulk milk samples as well as individual milk samples. For detecting antibody titres in bulk milk samples the most recent estimates of sensitivity and specificity by this method are 97.0% and 62.0% respectively (Sargeant *et al.,* 1997a). The testing of bulk milk samples is convenient and accurate as a screening test for BLV eradication programmes and once a herd is identified as BLV-positive on a bulk milk ELISA test, then further testing at the individual animal level is indicated.

Since monoclonal antibodies have been developed to enhance the sensitivity and specificity of ELISA test in the past decades (Mammerickx *et al.,* 1984) (Onuma *et al.,* 1985) (De Boer *et al.,* 1987) (Florent *et al.,* 1988) (De Boer *et al.,* 1989) (Nguyen *et al.,* 1993) (Sargeant *et al.,* 1997a) this technique has proved to be practical in terms of accuracy, cost, and ease of application. Thus, ELISA testing is the recommended diagnostic technique for preliminary surveys or for estimating the feasibility of a control programme, particularly for surveying herds those herds that have been declared BLV free (Nguyen *et al.,* 1993), (Hayes *et al.,* 1997).

BLV ELISA tests of cows sampled during the periparturient period (approximately two to six weeks before and after parturition) should be interpreted with caution. The shift of antibodies from the dam's circulatory system into the colostrum (which gives rise to false-positive test results in calves that consume that colostrum) may cause a false-negative test result for the dam (Johnson *et al.,* 1991a).

Nucleic acid-based tests

Detection of integrated proviral DNA using the Polymerase Chain Reaction (PCR) technique has been proposed as an alternative to serological methods in situations where it is essential to detect all infected animals. In terms of sensitivity, this test allows the detection of 1 BLV-infected cell in 1 x 10^4 bovine lymphocytes (Agresti *et al.,* 1993) and it is regarded as the most sensitive BLV test currently available (Evermann *et al.,* 1997). PCR allows the detection of BLV infection in cattle several weeks before antibodies are able to be detected by other methods (Kelly *et al.,* 1993) (Klintevall *et al.,* 1994). As a result of these advantages, PCR is the recommended diagnostic method when other serological tests have yielded doubtful results. A situation where this test may be preferred to others is in confirming that no transplacental transmission has occurred in valuable calves with BLV-positive dams.

Sensitivity of PCR was reported differently in moderate and low incidence herds. When PCR was evaluated in moderate incidence, it yielded a 10% higher number than ELISA and a 17.7% higher number than AGID. On the other hand, when PCR was evaluated in herds with incidence less than 5%, 43 of 52 provirus positive cattle (83%) were correctly identified by ELISA while only 37 of 52 (71%) PCR positive animals were corrected identified. This revealed a concordance of 73.4% for PCR and AGID and 81.1% for PCR and ELISA (Fechner *et al.,* 1996). A similar finding was reported by Reichel *et al.* (1998) when PCR was used to compare AGID, ELISA test kits and electrophoretic immunoblotting (EIB) in 399 field serum samples of which the average prevalence was less than 10%. The result showed that ELISA tests detected about 10% more reactors than the AGID and the EIB combined (Reichel *et al.,* 1998). The main reason for this discrepancy is probably due to the principle of selecting animals to be tested. Some BLV-seropositive animals may have virus sequestered in lymphphoid tissues which can be detected only by antibody-based techniques (Evermann *et al.,* 1997).

The use of PCR testing for BLV diagnosis has raised new insights into the aetiological agent of this disease. A trial was undertaken to compare antibody-based testing techniques, namely AGID and ELISA and nucleic acid-based testing techniques, namely PCR (Jacobs *et al.,* 1992). Southern blot hybridization was used to confirm the presence of DNA as well. In a naturally infected herd (n = 73), PCR was found to detect the tumour DNA in 4 of 10 EBL cases whereas it could not detect the tumour DNA in 5 of 27 adult cattle with lymphoma. Serological tests (AGID and ELISA) and PCR test results showed a concordance rate of 83.6%. Discordant test results occurred with approximately equal frequency between serologically positive and PCR negative (9.6%) and serologically negative and PCR positive (6.8%) groups. Since the BLV provirus appeared differently in some cases as mentioned earlier, conclusions from this study have suggested the role of BLV as an aetiologic agent in the development of EBL should be re-examined.

A summary of the important details of each of the diagnostic tests available for BLV detection are shown in Table 2-2.

Post mortem findings

In EBL tumour masses may be found in any organ. In cases of Sporadic Bovine Leukosis the most common sites of neoplasia are the kidneys, thymus, liver, spleen, and peripheral and internal lymph nodes.

Treatment

Radostits *et al.* (1994) has documented temporary remission of tumours associated with EBL by using nitrogen mustard or triethylenemelamine. A variety of other treatments has been attempted including the administration of adriamycin entrapped in liposomes conjugated with monoclonal antibody against tumour-associated antigens of bovine leukemia cells and certain immunopotentiators (Onuma *et al.*, 1989b), all with disappointing results. Currently there is no therapy recommended for treatment of animals with EBL (Radostits *et al.*, 1994).

Epidemiology

Most early descriptive studies of the epidemiology of EBL and BLV infection are either cross-sectional or prospective cohort studies involving small numbers of cattle herds in which sampling techniques were either non-randomly assigned or not described. As a result of these deficiencies reliable inferences about the population from which the animals were chosen are not able to be made (Johnson *et al.*, 1992).

Descriptive studies

Geographical distribution of BLV infection

BLV infection has been identified in all continents of the world, however, the prevalence of the infection varies markedly between and within countries.

In the USA, no nationwide surveys have been completed, but the most recent estimates suggest that 22% of the adult cattle population are seropositive to BLV (cited in (The New Zealand Dairy Industry 1997). An investigation by the National Animal Health Monitoring System (NAHMS) investigated the prevalence of BLV infection in randomly selected dairy operations in 20 states of the United States (Anon 1998). This study revealed that 89% of herds had at least one animal that was seropositive for BLV. Prevalence of infection varied geographically with regions in

the south-east having up to 99% of operations with at least one animal seropositive to BLV. Within-herd seroprevalence was at least 25% in 75% of the positive herds. Other high prevalence areas were the west, the mid-west and the north-east. In a similar study of beef herds conducted by NAHMS in 1997 (Anon 1999a) it was estimated that approximately 38% of all beef operations had at least one animal that was seropositive to BLV and 10.3% of all beef animals tested were seropositive. For beef herds, the highest prevalence regions were the north and south central states, the south-east and the north.

In Canada serological surveys have shown BLV prevalence to be concentrated in the provinces of Manitoba, Ontario and Quebec. In these regions 40% of dairy herds and 11% of beef herds have at least one animal seropositive to BLV. The magnitude of BLV infection in dairy cattle was eighteen times higher than in beef cattle (9.3% *vs*. 0.5%) (Reed 1981), (Heald *et al.,* 1992).

BLV control programmes have been established in member countries of the European Community (EC) since 1980. A 1987 report stated that the proportion of the cattle population seropositive to BLV was between 0.5 - 1.5% (cited in (Johnson *et al.,* 1992). Of the EC member states, only the Netherlands and Denmark claim to be completely free of BLV infection.

In Australia, prevalence of BLV infection varies between states. The high prevalence was reported in Queensland and New South Wales with approximately 15% of dairy herds estimated to be infected (Anon 1999c). The highest prevalence was detected in 13.7% of dairy cattle in Queensland (Office International des Epizoooties 1992).

Limited serological surveys have been reported in South Africa, South America and Asia. Estimates of the proportion of cattle seropositive to BLV in Africa are as follows: West Africa 37%, Guinea 10%, and Zimbabwe 20% (Schwartz *et al.,* 1994). In eastern countries, BLV has been reported in Japan (Onuma *et al.,* 1980) (Kono *et al.,* 1983),Taiwan (Wang 1991), Israel (Brenner *et al.,* 1986) and Saudi Arabia (Hafez *et al.,* 1990). The world-wide distribution of reported BLV infection is shown in Figure 2-6.

Details of serological surveys of BLV prevalence conducted in New Zealand are shown in Table 2-3.

By far the most comprehensive and thorough study of BLV prevalence in dairy cattle in New Zealand has been that conducted as part of the EBL Control Scheme initiated in 1996 by the New Zealand Dairy Industry and the Livestock Improvement Corporation. Between 1996 and 1997 herd prevalence in dairy cattle was estimated to range from 12.8% in the South Island to 2.2% in Taranaki region (Figure 2-7).

Follow-up blood testing conducted in November 1998, as part of the EBL Control Scheme has shown that 889 of 14336 herds tested (6.2%) contain at least one animal seropositive to BLV. Of the 889 BLV-positive herds only 26 have been shown to have a within-herd prevalence of greater than 10%. In total 5557 of 324971 animals sampled were seropositive to BLV (1.71% prevalence) (Hayes 1998).

Breed and production type

Random sampling surveys conducted in North America have estimated the prevalence of BLV infection in dairy cattle to be 2.2 - 29 times that of beef cattle (Burridge *et al.*, 1981) (DiGiacomo 1992a), (Hopkins *et al.*, 1997).

The earliest descriptive epidemiological study in which there were unbiased estimates of dairy and beef cattle BLV prevalence was a cross-sectional study conducted in Florida (Burridge *et al.*, 1981). Eighteen dairy and 28 beef herds were selected from four geographical regions by stratified random sampling procedures. This study revealed that there was higher antibody prevalence in Jersey cattle ($P < 0.01$) compared to other breeds (Holstein, Guernsey, and Brown Swiss). Also, Jersey cattle tended to become infected at an earlier age than Holstein cattle. In another study Kaja *et al.* (1984) found no difference among five breeds (Ayrshire, Guernsey, Holstein, Jersey and Brown Swiss) and DiGiacomo (1992a) and Lassauzet *et al.* (1991b) reported similar results. Thurmond *et al.* (1982b) and Thurmond *et al.* (1983c) found that both the prevalence of BLV-infected animals and the incidence of BLV infection varied significantly between Jersey or Holstein cattle, and that within each breed prevalence and incidence of infection were strongly influenced by age (DiGiacomo 1992a).

Age, sex and parity

Transmission rates of BLV have been shown to depend on age. In dairy cattle, infection rates are low in calves less than 6 months old and increase thereafter to

about 3 years (Olson *et al.*, 1978), (Burridge *et al.*, 1981). After three years of age, there is some disagreement in the literature regarding the rates of infection. Cross-sectional studies have indicated that infection rates remain fairly stable (Wilesmith *et al.*, 1980) while longitudinal studies indicate a declining infection rate with age (Huber *et al.*, 1981a), (DiGiacomo 1992a). Only 0.5 - 0.8% of BLV transmission occurs vertically (Johnson *et al.*, 1991a) (Hopkins *et al.*, 1997).

Genetic factors

The finding that lymphosarcoma and persistent lymphocytosis aggregate along familial lines was suspected during an EBL outbreak in US in the 1970s (Ferrer 1980) and experimental studies subsequently have confirmed this hypothesis. In Denmark Bendixen (1965) observed the development of lymphosarcoma in 25 leukosis-free herds in which cattle from high-incidence herds had been introduced. Lymphosarcoma appeared in the offspring of the imported animals as well as in the original stock of the herds (cited in (Ferrer 1980). Thurmond *et al.* (1982c) and Thurmond *et al.* (1983a) demonstrated no significant association was found between the BLV status of the dam and BLV infection in progeny of uninfected cows. It was suggested that the difference may have been attributed to the high prevalence of infection in certain herds, rather than a genetic effect.

Molecular epidemiological studies have identified that the genetic susceptibility to BLV infection depends on the type of Bovine Lymphocyte Antigens (BoLA) present in the individual. Shorthorn cows with BoLA-w8 specificity have been shown to be at an increased risk of becoming seropositive to BLV following exposure to the virus. Resistance to persistent leukosis among BLV seropositive cattle is associated with BoLA-DA7, whereas susceptibility to persistent leukosis is thought to be associated with BoLA-DA12.3 (Lewin *et al.*, 1986).

Herd size

There are conflicting reports in the literature regarding the association of herd size and within-herd prevalence of BLV infection. Olson *et al.*(1975), in a study of 112 herds found that high BLV prevalence was associated with small herd size (less than 50 cows), whereas more recently Johnson *et al.* (1992) and Casal *et al.* (1990) have found that larger herd sizes are associated with higher within-herd BLV prevalence.

Recently the NAHMS (Anon 1998) survey of subsets of the US dairy cow population have produced estimates of herd-level BLV infection and animal-level BLV infection in relation to herd size (Figure 2-8). In these surveys, herd-level BLV infection and animal-level BLV infection increased with herd size. Stocking density (that is, the number of animals per hectare of farming land) is suspected as being a more efficient indicator of the risk of BLV infection compared to herd size per se.

Season

There have been conflicting reports regarding the seasonal pattern of BLV infection (Bech-Nielsen *et al.,* 1978), (Onuma *et al.,* 1980) (Wilesmith *et al.,* 1980), (Thurmond *et al.,* 1982b), (Lassauzet *et al.,* 1991b). Fluctuations in disease incidence were reported to be preceded by a similar cyclical pattern of blood-sucking insect population in high vector-density areas. Another trend was found in seasonal calving production, when heifers came back from pasture and were brought into the herds for calving. Thus, these findings presumably resulted from the differences in the type of calving, type of housing and management events in the studied herds.

A summary of the key epidemiological features of BLV infection in cattle is shown in Table 2-4.

Risk factors for BLV transmission

Since BLV is integrated within the genome of lymphocytes within the host, there is virtually no virus found "free" in the body. Thus, transmission of the virus will only occur where there is the transfer of blood from infected to non-infected animals. Between herds, major risk factors for the introduction of the virus into herd is the movement of infected animals into the herd. Within herds, risk factors for the transmission of BLV infection from one animal to another tend to be age-specific and related primarily to farm management practices.

The next section of this review will examine specific risk factors for BLV transmission, in order of importance.

Stock purchase

The impact of purchasing stock has been demonstrated in a case study described by (DiGiacomo *et al.*, 1986b). In this study a herd made up of 265 cows increased over a period of nine years to 485 cows. Three years after the commencement of BLV monitoring, the seroprevalence of BLV reduced from 23% to 11%. The incidence of infection was estimated to be 1 - 2 % of the herd per year. During year four a herd of 99 cows acquired from a single source was introduced into the established herd. The seroprevalence in the introduced herd was 28% and, after mixing with the established herd, the incidence within the established herd increased to 7 - 10% per year. These findings suggest that the introduction of (untested) stock into (closed) herds carries a risk of increasing the incidence in the recipient herd.

Serostatus of the dam

Ferrer *et al.* (1976) was the first to report vertical BLV transmission under field conditions demonstrating that both virus and antibody could be detected in calves born to BLV-positive dams in the absence of any other source of exposure to the virus. Rates of vertical transmission have been reported in the literature. Stärk (1996) estimated rates of between 15 to 20% whereas more recently Thurmond *et al.* (1983b); Thurmond *et al.* (1983c) estimated vertical transmission rates of between 0.5 and 8.0%. A survey of dairy cattle in New Zealand in 1996 (The New Zealand Dairy Industry 1997) found that 11% of calves born to infected dams had evidence of BLV infection compared to 2.8% in calves born to BLV-negative dams.

A three-year prospective study involving 143 calves born from BLV-infected cows was undertaken to evaluate the likelihood of vertical transmission of BLV. Risk factors for vertical transmission were high prevalence herds, the presence of maternal lymphocytosis ($>1.2 \times 10^5$ cells/μl, $P = 0.04$), and the presence of EBL in the dam (Lassauzet *et al.*, 1991b). Presence of clinical EBL in the dam showed the highest relative risk of transmission (RR 46.0, $P < 0.01$) (Lassauzet *et al.*, 1991a).

Colostrum

BLV has been found in both colostrum and milk from BLV-infected cows (Miller *et al.*, 1979), (Straub 1984). Changes in the concentration of maternal antibodies around the time of calving and changes in the concentration of antibodies in the calf

around the time of birth are shown in Figure 2-9. The susceptibility of calves to infection via colostrum or milk is modified by the level of colostral antibodies to BLV and the calf's age. Both observational studies and clinical trials have been conducted to investigate this risk factor with conflicting results identified (van der Maaten MJ *et al.,* 1978b), (van der Maaten *et al.,* 1981b) (Thurmond *et al.,* 1982b), (Thurmond *et al.,* 1983c) (Lassauzet *et al.,* 1989a). Mussgay *et al.* (1980) reported that calves fed with milk following colostral feeding were five times more likely to be infected with BLV compared to those fed with a milk substitute when tested at 6 to 24 months of age (cited in (DiGiacomo 1992b). In another interventional study where bulk milk feeding was stopped and calves were individually fed with milk from BLV-free cows, no calfhood infections occurred up to weaning (Dimmock *et al.,* 1991).

Management

High rates of BLV transmission occur when young calves are exposed to the practice of gouge dehorning when the dehorning instrument was not cleaned or disinfected (Darlington *et al.,* 1984) (Lassauzet *et al.,* 1990) (DiGiacomo *et al.,* 1990). The relative risk of BLV infection in calves dehorned under these conditions was 6.0 times that of calves not dehorned at all (DiGiacomo *et al.,* 1985).

The risk of BLV infection attributable to dehorning was 77% in heifers dehorned after BLV positive calves while it was reduced to 38% in heifers dehorned after BLV negative calves. In terms of the risk due to different methods of dehorning, groups of heifers that were placed in pens holding gouge-dehorned heifers were 6.6 times more likely to develop BLV infection than that of groups of heifers that were placed in pens without gouge-dehorned heifers (Lassauzet *et al.,* 1990).

In support of these procedures being important means by which BLV is transmitted, intervention studies aimed at changing these risk-prone management practices have been shown to successfully reduce the prevalence of BLV in individual herds. In a closed 600 cow Washington dairy herd, changing the dehorning method to aseptic electrical disbudding (as the single means of controlling for BLV transfer) resulted in prevalence reducing from 68% to 40% in 3 years (DiGiacomo *et al.,* 1987). In another study of a 163 cow Californian dairy herd, modifying the method of

dehorning reduced the prevalence of BLV infection from 80% to 4% in replacement heifers (Lassauzet *et al.*, 1990).

While experimental work has been largely directed at examining the effect of dehorning on the spread of BLV it is hypothesised that similar precautions applied to the conduct of other minor procedures such as tail docking, castration, the injections of medications and supernumary teat removal could reduce the risk of horizontal transmission of the virus.

Rectal examination

In this discussion, the term rectal examination describes the act of rectal palpation for the purposes of pregnancy diagnosis, ultrasonography, artificial insemination and embryo transfer.

In the course of modern dairy management milking cows receive multiple rectal examinations annually. In seasonally-calving herds large groups of cows are assembled on occasions to be inseminated and to have the presence of pregnancy determined by a veterinarian. Under this system of management the use of common rectal palpation sleeves and vigorous palpation may permit the transfer of blood from one animal to another and therefore permit the spread of BLV infection within the herd.

Both experimental and observational studies have been carried out to assess the potential of BLV spread via this route. Initial experiments demonstrated the infectivity of BLV by infusing 500 mL of BLV-infected blood into the rectum of BLV negative animals (Henry *et al.*, 1987). All animals in the study seroconverted within 5 weeks after treatment.

An observational study was conducted in a commercial dairy and in a veterinary teaching unit over 20 months to assess the effect of using common rectal palpation sleeves (Hopkins *et al.*, 1991). It was found that the use of common sleeves was not a risk for transfer of BLV in the commercial farm but was a risk factor in the teaching unit, increasing the risk of infection by a factor of 8.3 ($P < 0.02$). The increased risk of BLV infection in the teaching facility was thought to be a result of the increased trauma to the rectal tract caused during the process of teaching rectal examination to veterinary students.

In a cohort study conducted in a commercial dairy herd, cows palpated in the standard manner without sleeve change were 2.8 times more likely to develop BLV infection (95% CI 1.1 - 6.8, $P < 0.02$) (Divers *et al.*, 1995).

Another cohort study was undertaken in a California dairy to estimate the risk of rectal palpation with respect to the prevalence of infection. The probability of seroconversion within 3 months after rectal palpation was reported due to the prevalence of infection in cows at the time of palpation (Lassauzet M. L. *et al.*, 1989b). No significant difference in probability between the two groups was shown by logistic regression analysis. It was concluded that BLV transmission by rectal palpation was uncommon. Alternatively, the results of this study might indicate that the risk of transmission was unrelated to the prevalence of infection (Hopkins *et al.*, 1997) because incidence occurred in both groups and there were no non-infected group as a control in this study.

Risk factors associated with calving

In seasonally calving systems, non-infected cows are at risk of being exposed to the blood, tissues and uterine fluids of BLV-positive cows at the time of parturition. To examine the effect of calving management on risk of BLV infection, a case control study was conducted in 268 Ontario dairies (Heald *et al.*, 1992). The odds ratio of infection in cows calving in separate calving pens in winter (compared to other locations) was 3.4 (95% CI 1.7 - 6.9). The odds ratio of infection in cows calving in separate pens in the summer (compared to calving elsewhere, mostly at pasture) was 0.4 (95% CI 0.2 - 0.9). The interpretation of these findings is that cows calved at pasture would have greater access to each other as well as to contaminated discharges during summer time. Alternatively, in winter time most calving cows were confined in tie-stalls which were more likely to get contaminated than other places.

A matched case-control study was carried out on a commercial Holstein dairy to determine the risk of developing BLV infection between periparturient period and non-periparturient period. The risk of cows acquiring infection during periparturient period was 6.0 times (95% CI 1.7-21.5) more than seronegative cows (20 seroconverted cows *vs* 68 uninfected cows) (Pollari *et al.*, 1993).

Stocking density

Several longitudinal studies have formed the hypothesis that close physical contact as well as intensive management practices are associated with the incidence of BLV infection.

In an observational study conducted in one Californian dairy the risk of BLV transmission was 2.9 times higher in non-pregnant cows compared to pregnant cows and the risk of infection significantly increased as prevalence of BLV infection in a pen increased. Cows in a pen in which 60 percent of cattle were infected were 4.7 times more likely to become infected than were animals in a pen in which 20 percent of animals were infected (Lassauzet *et al.,* 1991b). A possible explanation for these findings is that the oestrous behaviour of non-pregnant cows provided a regular opportunity for close physical contact and opportunity for BLV transmission.

In another prospective observational study in a Californian dairy, cows were 4.7 times more likely to become infected if in a pen where the BLV prevalence was 60% compared to a pen where 20% of cows were infected ($P < 0.05$). In this study it was also found that the relative risk of infection for heifers increased by 6.6 times for those housed in pens with 50 - 70% BLV prevalence compared to those housed in pens with 10 - 30% prevalence ($P < 0.01$) (Lassauzet *et al.,* 1991b). Interpreting the literature that is available it is evident that the contribution of close physical contact to BLV infection risk is variable, with higher risks being associated with high BLV prevalence and/or management practices that facilitated the transfer of blood from one cow to another.

Vaccination

Under field conditions, BLV-contaminated vaccine has only once been reported as a cause of high infection rates. In a case study in a large Queensland herd involving contaminated Babesiosis vaccine (Rogers *et al.,* 1988) the infection rate in vaccinated dairy cattle was 62% (158 of 255) compared to 6.1% (35 of 575) in unvaccinated cattle in the same herd.

Other studies have reported that vaccination (particularly Brucella vaccination) was not an effective means of BLV transmission (Lassauzet *et al.,* 1990), (Thurmond *et al.,* 1983c). Experimental contamination of a tuberculin needle with BLV-positive

blood has resulted in infection, however administration of the intradermal tuberculin test under production conditions has not resulted in BLV transmission (Roberts *et al.,* 1981a). Thus, it is suggested that gross contamination of blood in subcutaneous, intradermal or intramuscular injection under natural conditions carries low risks of BLV transmission.

Ear tagging

An experiment to evaluate the role of tattoo dyes and pastes in spreading BLV infection was conducted in sheep (Lucas *et al.,* 1985). Sheep were tattooed immediately after the tattooing of a BLV-positive calf. The results of this experiment showed that 88% of the 24 treated sheep and all animals in the control group seroconverted within five weeks of tattooing. Thus, tattooing could be a means of disease spread and tattoo dyes could not prevent BLV transmission either. Similar studies using cattle as recipients have not been carried out. Nevertheless, the introduction of disease spread by this mean did not appear under field conditions (Lassauzet *et al.,* 1990), (Thurmond *et al.,* 1983b).

Insect vectors

Biting insects and arthropods are postulated to be a significant potential vector for BLV spread as a result of their ability to transfer blood from one animal to another. In the literature three study designs have been applied to test this hypothesis: (1) those in which insects feed naturally on infected donor cows or artificially on BLV-infected blood and the mouth parts or midguts of the insects are harvested and inoculated into BLV-negative cattle or sheep; (2) those in which insects feed naturally on both infected donor cows (sometimes with interruption of feeding) and susceptible cattle; and (3) ecologic or observational studies examining the effect of seasonality of infection (Hopkins *et al.,* 1997).

Results of studies conducted using the first study design have demonstrated high rates of transmission in sheep (Ohshima *et al.,* 1981), (Buxton *et al.,* 1982) (Kaaden *et al.,* 1982), (Buxton *et al.,* 1985) (Weber *et al.,* 1988), (Perino *et al.,* 1990), and lower rates in cattle (Buxton *et al.,* 1985), (Foil *et al.,* 1989) (Hasselschwert *et al.,* 1993).

In terms of seasonal pattern of infection, several investigators have reported increased BLV incidence in the summer and autumn months, suggesting an association with peak insect populations (Bech-Nielsen *et al.,* 1978) (Ohshima *et al.,* 1981) (Manet *et al.,* 1989), whereas others have found no seasonality (Lassauzet *et al.,* 1991b) (Sprecher *et al.,* 1991).

The significance of vector transmission under field conditions remains controversial. The conclusion from the experimental data available is that the transfer of BLV is dependent on: (1) the quantity of blood retained on the mouth part of insects after feeding, and (2) characteristics of the feeding vector. Larger, engorging feeders (such as tabanid flies), in contrast to the finicky feeders (such as midges and mosquitoes) and vectors which frequently alternate hosts are considered to be more successful vectors for BLV transmission. The important role for stable flies, horn flies and tabanids in the transmission of BLV may be limited to local areas of heavy fly infestation whereas painful bites from insects may cause interrupted feedings on infected cattle and movement of vectors to susceptible cattle. Nonetheless, this may be subject to the prevalence of the herds as well.

Vertical transmission

Studies have found that semen from BLV-infected bulls does not normally contain the BLV unless the semen itself is contaminated with lymphocytes (Miller *et al.,* 1979) (Kaja *et al.,* 1982) (Monke 1986). Only one study, where semen was collected by transrectal massage, has demonstrated the infectivity of BLV via semen (Lucas *et al.,* 1980). Similarly, ova and embryos from BLV-infected cows have not been found to contain BLV (Eloit M *et al.,* 1986) (Eaglesome *et al.,* 1982) (Kaja *et al.,* 1982) (Kaja *et al.,* 1984) (DiGiacomo *et al.,* 1990). Both experimental and field studies indicate that breeding by natural service, artificial insemination or embryo transfer is not a significant vehicle for BLV transmission (Miller *et al.,* 1979) (Kaja *et al.,* 1982) (Roberts *et al.,* 1982a) (Thurmond *et al.,* 1983b), (Thurmond *et al.,* 1983c). Only one study has reported seroconversion after insemination using semen from BLV-infected bulls (Belev *et al.,* 1986). The results of these studies indicate that artificial insemination is not a risk for BLV transmission under field conditions. However, it is conceivable that artificial insemination could result in BLV transmission if multiple cows are bred using shared AI equipment or common

sleeves for rectal palpation during the AI procedure, a common practice under New Zealand dairy farming conditions.

The risk BLV via embryo transfer appears to be very low providing that proper techniques are used (Bouillant *et al.,* 1981) (Kaja *et al.,* 1982) (Kaja *et al.,* 1984) (DiGiacomo *et al.,* 1986a; DiGiacomo *et al.,* 1990).

Cross species transmission

BLV occurs primarily in domesticated cattle of the species *Bos taurus.* Infections have occurred in other bovine and non-bovine species as a result of both natural and experimentally-induced infections. No evidence of transmission of BLV from other species to cattle has been reported (Johnson *et al.,* 1992).

A summary of the relative importance of BLV transmission risks is shown in Table 2-5.

Economics

Losses as a result of EBL-BLV are both indirect an direct. Direct costs are those associated with disease: lost production in cattle with clinical manifestations of EBL, veterinary costs associated with treatment and diagnosis of EBL, and replacement costs associated with death or culling. Indirect costs are costs associated with loss of revenues due to restrictions imposed on the export of cattle and cattle products such as semen and embryos and costs to society for maintenance of regulatory agencies and research (Pelzer 1997). Thurmond (1987) estimated that in 1984 the presence of EBL-BLV in North America cost US $44 million. This comprised US $1.6 million for lost milk production, US $40.5 million for the cost of replacement of cattle with lymphoma, US $0.5 million for veterinary services and US $1.7 million associated with restrictions placed on foreign trade of semen and embryos.

Direct costs

Clinical disease

Direct losses from EBL are associated with death and involuntary culling of stock. Since animals are condemned at slaughter they have little or no salvage value. The

main costs associated with cases of EBL are the cost of replacements, the loss of production associated with premature culling, and if the cow was pregnant at the time of removal, foetal wastage.

Recent reports for 1986 - 1990 state that the mean number of cattle condemned for EBL in federally inspected slaughter plants in the United States was 13684 (Hubbert *et al.,* 1996) compared to 11367 in 1984 (Thurmond 1987). The condemnation rate due to malignant lymphoma was 4.2 per 10000 carcasses examined at slaughter. If these cattle incurred an average replacement cost of US $1200, the estimated cost of replacing EBL affected stock was US $16.5 million. This estimate is based on an assumption that only half of the malignant lymphoma cases are observed at an inspected slaughter facility and does not include other costs (Thurmond *et al.,* 1985b).

Milk production

While it is well established that clinical lymphosarcoma causes a dramatic decrease in milk production (Radostits *et al.,* 1994), the effect of seroconversion to BLV on milk production has not been clearly identified. An early case-control study showed that seropositive cows had slightly longer lactations resulting in more milk production in the first two lactations compared to seronegative cows ($P < 0.05$) (Langston *et al.,* 1978). A retrospective longitudinal study conducted later found that there were no significant differences in milk production between BLV-positive and BLV-negative cows (Huber *et al.,* 1981a). In contrast, a matched case control study revealed that a BLV-positive cow produced a total of 3.5% less milk and had a mean of 48 more days open than did a BLV-seronegative cow (Brenner *et al.,* 1989). Jacob *et al.* (1991) reported the same finding as Huber (1981) but after adjustment for herd size, days in milk and age the association between serostatus and production was no longer present. A longitudinal study showed that, in the lactation of removal, BLV seropositive and cows with clinical EBL were higher producers than seronegative cattle in the cull lactation. As an increase of BLV seroprevalence with age has been reported in many studies, even if cows are not grouped by production levels, BLV infection might be concentrated in cows with high production due to their age distribution (Pollari *et al.,* 1992).

Estimations of costs associated with BLV infection may be confounded if high milk production, or the stress associated with production, is found to be a factor predisposing to BLV infection and/ or the expression of persistent lymphocytosis or lymphoma. This potential bias necessitates within-cow study designs which would permit the analysis of milk production changes for individual cows as they pass from the being BLV-seropositive to persistently lymphocytotic to developing clinical EBL (Thurmond 1987).

Culling and replacement

Distinct from culling related to EBL, the association of seroprevalence and culling has been investigated in many studies. In a herd of 184 cows with 45% BLV seroprevalence, transformed age-specific culling rates for seropositive and seronegative cows were predicted using regression equations. This study showed that 10 BLV-positive cows were culled in excess of the expected number computed for BLV-negative cows (Thurmond 1984). Culling rates in BLV-infected herds were also higher than were in BLV-free herds (Emanuelson *et al.,* 1992). A criticism of these studies is that no direct reasons for the increase in culling rates has been documented. It is suggested that BLV infection and culling may be independent of each other but share a common factor that influences the occurrence of infection as well as culling. As there is diversity in management practices and geographical areas, the effects of BLV infection on culling should be determined based on specific farm situations rather than making generalisations applicable to the industry as a whole.

Losses for EBL are expected to vary between herds according to prevalence of BLV infection and the rate of lymphoma among infected cattle. The cost of replacing culled cows might be high if there is a limited supply of replacements under conditions of high demand. In the estimation of 40500 EBL cattle in the US in 1987, subject to the assumption of constant market price for replacements, cost of replacing dairy cattle afflicted with lymphoma would reach US $40.5 million annually (Thurmond 1987).

Diagnosis of clinical EBL cases

The direct cost of veterinary services depends on the stage of BLV infection. Early in the clinical course of EBL, where laboratory work is required for a definitive diagnosis, the costs tend to be higher.

Indirect costs

Loss of revenue

Due to the requirement of a negative serologic test prior to export BLV may provide an international trade barrier for the exportation of breeding animals, semen, and embryos. Based on the North American figures for 1995, 8387 beef and 14380 dairy breeding cattle valued at approximately US $30.5 million were exported to various foreign countries. Semen exported in the same year was valued of US $67 million (United States Department of Agriculture 1995). If importing countries insisted on sourcing stock and genetic material from BLV-free areas, the loss of these markets would have a significant effect on the US cattle industry.

Cost of control and eradication

The cost of control and eradication varies from farm to farm and depends on which strategy is adopted. Three basic approaches are concerned with: (1) test and slaughter, (2) test and segregation, and (3) test (monitor) and implementation of preventive management. Two factors affecting the cost of a programme are the prevalence of infection at the start of the programme and the value of the animals. This cost will vary from one country to another. Cumulative costs of EBL control in Germany from 1967 to 1987 were 1119 million DM (Kautzsch *et al.*, 1990) (~ NZ$ 1178 million). The cost per case was estimated at 3000 DM (NZ $3158) until 1983 and at 5000 DM (NZ $5263) after 1984 onwards.

Control and eradication

Done (1985) defined control as the reduction of the morbidity and mortality from disease and as a general term embracing all measures intended to interfere with the unrestrained occurrence of disease, whatever its cause. Control can be achieved both by treating animals already afflicted with the disease which reduces its prevalence

and by preventing establishment of disease which reduces both its incidence and prevalence. The term 'eradication' has been defined in four different ways, namely: (1) the extinction of an infectious agent from a localized area, (2) the reduction of infectious disease prevalence in a specified area to a level at which transmission does not occur, (3) the reduction of infectious disease prevalence to a level at which the disease ceases to be a major health problem and, (4) the regional extinction of an infectious agent (Thrusfield, M 1995).

Several factors must be considered before either a control or an eradication campaign for a given disease can be undertaken. These include (Thrusfield, M 1995):

1. The level of knowledge about the cause of the disease and, if the disease is infectious, the level of knowledge about its transmission and maintenance, including host range and the nature of the host/ parasite relationship;
2. The veterinary infrastructure within the region of interest;
3. The ease with which the disease is diagnosed and how surveillance of the disease is to be undertaken;
4. The availability of replacement stock;
5. Producers' and society's views of control/eradication of the disease;
6. The disease's public health significance
7. The existence of suitable legislation with provision for compensation;
8. The possible ecological consequences of control/eradication;
9. The economic costs and the availability of funds for the control/eradication programme.

The success of a control programme depends on two issues: (1) the ability to identify risk factors that predispose to infection (Evermann *et al.*, 1997) and (2) the ability to accurately diagnose the condition. The first issue enables better-targeting of economic resources whereas the second issue ensures maximum efficiency of diagnostic resources when they are applied. Ruppanner (1983), DiGiacomo (1992), and Pelzer (1997) have described three basic strategies for establishing disease control programmes: (1) test and removal of affected stock, (2) test and segregation

of affected stock and, (3) monitoring of the prevalence of disease and implementation of management strategies to reduce the transmission of disease.

Control strategies

Test and removal

The strategy of testing and removing BLV-positive is suitable in areas of low prevalence of infection because the expense for indemnity and the support programme is not high. Test and removal is not a preferred strategy in herds with animals of high value or of high genetic potential. By eliminating all positive animals and establishing a BLV-free herd the producer no longer incurs the cost of testing nor the costs associated with a control programme.

In most studies that examined the efficiency of test and removal as a means of controlling, BLV herds were tested at intervals of one to six months. Herds with a lower seroprevalence of BLV infection required fewer tests to eliminate infected cattle (DiGiacomo 1992a). In almost all studies, seropositive cattle were eliminated from the herds after one, two, or three serologic tests, including the initial test to determine the prevalence of BLV infection in the herd (Evermann *et al.,* 1992b).

Test and segregation

The control method of test and segregation is based on the isolation of seropositive animals immediately after testing. The advantage of this method is that high-producing or genetically superior animals are to be retained within the herd. The disadvantages associated with this method are increased costs of housing, confinement, and pastures for infected animals as well as the added labour to take care of another group of animals separately within the same production unit. With this strategy costs tend to be high due to the frequent testing required to identify infected animals for segregation and the higher numbers of replacement animals that are required over a longer period of time. Also, the success of this strategy is dependent on physically separating infected and uninfected cattle and this goal may be difficult depending on individual farm circumstances (Evermann *et al.,* 1992b).

Numerous reports have demonstrated that this approach has successfully eradicated BLV infection from herds (Miller *et al.,* 1982a) (Shettigara *et al.,* 1989) (Sprecher *et*

al., 1991) (DiGiacomo 1992a) (Johnson *et al.*, 1992), (Monke *et al.*, 1992). As with the test and removal programme, segregating seropositive cattle from the herd eliminated BLV infection after one or two rounds of testing.

Test and implement control strategies

The basis of testing and implementation of control strategies is to raise negative replacements that will replace older, infected animals that leave the herd either due to natural attrition or selective culling (Ruppanner. R *et al.*, 1983) (Thurmond 1991b). The advantage of this strategy is that neither have the infected animals to be removed at the time of identification nor are there costs involved with segregation. The disadvantages are the difficulties of implementation of changes to routine practices. Also, due to the slowness of progress the producer may lose enthusiasm for the control programme, resulting in laxity. The time taken to control and eliminate infection from the herd is dependent on the initial prevalence of infection, the culling rate, the success of raising non-infected replacements, and the farm personnel's commitment to the programme (Lassauzet *et al.*, 1991b). The cost of this program depends on both frequency of testing and the cost of changing management practices.

Eradication strategies

The Office International des Epizooties (OIE) has determined guidelines for the declaration of a disease-free status (Anon. 1993). Countries declaring themselves free from BLV should satisfy the requirements of OIE for at least three years, that is: (1) at least 99.8% of the country's herds are classified as BVL free and (2) a random sample of the country's herds should be taken with the sample size sufficient to provide a 99% level of confidence of detecting EBL or BLV infection if it is present at a prevalence rate exceeding 0.2% of the herds.

To achieve eradication at a national level, the following approach is suggested (Miller *et al.*, 1982a):

1. Establish herd BLV status

- test and identify BLV-positive animals
- manage BLV-positive animals (as outlined above)

2. Maintaining BLV-free status in those declared BLV-free

– test all animals bought into the herd

– institute surveillance testing

Eradication at the herd level

The earliest documented attempt to eradicate BLV infection from a herd (without eradication at the national level) was conducted on a large stock farm at Kitasato University in Japan (Yoshikawa *et al.,* 1982). In this case, testing and removal of BLV positive stock was used as well as maintaining a closed herd. Of 227 cattle with a prevalence of 3.52%, testing was performed approximately every three months on nine occasions. No more new cases were found after three years.

Johnson *et al.* (1985) reported the control of BLV infection in a 114 cow dairy herd in Michigan, USA. Ninety-five percent of the cows were positive for BLV antibodies using AGID. The BLV seronegative animals were examined monthly for BLV antibodies starting at 6 - 7 months of age. The reactor animals were separated from the seronegative animals when the reactors had 2 positive AGID tests consecutively. Following a 3-year complete separation of positive animals from negative animals, the overall point prevalence decreased from 95 to 34%. The percentage of BLV-positive animals in 4 age groups, namely 6 - 15, 16 - 23, 24 - 47 and older than 48 months, decreased from 19 to 17%, 58 to 14%, 90 to 33% and 100 to 90%, respectively. Possible explanations of the high incidence persisting in the lactating cows were: (1) a high prevalence in the older cattle and a large number of carriers available to infect the younger cattle at risk and (2) noncompliance to anti-BLV management strategies (Evermann *et al.,* 1987).

In Canada, nine herds consisting of 867 cattle were studied for a control programme using test-removal and test-segregate measures (Shettigara *et al.,* 1986). These herds were not closed and the replacement cattle were isolated and serotested immediately before and after purchase. Contact of experimental animals with animals from other herds was prohibited. Each entire herd was serotested at approximately six-month intervals for three years. Incident cases were immediately isolated at least 200m from the negative cattle and were culled within 30 days. The median of point prevalence rate for the first serotest was 22.0% and the median of incidence rate for

the succeeding eight serotests declined to 0.56%. The seropositive tests were attributed to naturally acquired infections. For instance, there were some cows incubating BLV during the initial serotest, and violations of the control protocol by the herd owners were failing to cull seropositive cows. The average expenditure incurred by Agriculture Canada for this project was US $1220 per seropositive animal. The success of this study was mainly attributable to the policy of indiscriminantly culling BLV positive animals.

A comparison of the effectiveness of control measures in two herds has been reported by Brenner (1988). In the first herd, where BLV prevalence was 8.4%, test and slaughter was used. In the second herd, where the prevalence was 30.8%, test and segregation was used. Test and slaughter achieved eradication within 12 months whereas test and segregation achieved eradication within 24 months.

Since viral antigen expression (VAE) in lymphocytes was found to be associated with BLV infection, this finding was also adopted as a means of controlling the spread of infection (Molloy *et al.*, 1994). Five commercial dairy herds containing between 126 and 304 cattle were tested twice using AGID to detect serostatus and using ELISA to detect VAE. Selective culling was based on VAE testing. All seropositive and antigen-positive animals were culled immediately while seropositive and antigen-negative animals were retained in the herds for at least 16 months. Control was almost achieved in four herds with the prevalence ranging 19 - 21% because only two incidences were detected within a year. This may have been a result of delayed VAE testing until some time after the initial test was completed. Nonetheless, in the fifth herd with highest prevalence (39%), VAE was done immediately after AGID test. Culling was based on the level of antigen expression in VAE testing (high, moderate and low) which took five months to cull most high and moderate VAE animals. A rapid reduction of the prevalence was reported but two new cases were still detected at this time. Eventually the eradication was accomplished in these herds in two years after implementation of control programme.

Eradication at a national level

BLV eradication programmes on a national basis were originally undertaken in Europe. The first official BLV control programme was introduced in Denmark in

1959 (Gottschau *et al.,* 1990). Under this scheme all adult cattle from herds in which incident cases of EBL were diagnosed were subjected to a haematological examination for the presence of a persistent lymphocytosis (defined by Bendixen, 1965 as having lymphocytosis present for at least two consecutive months). BLV positive herds identified in this manner were declared to be closed and indemnity was offered to induce the owners to have their entire herd slaughtered. The use of the haematological test was discontinued in 1979 when the AGID test was introduced and used up until 1982. A routine herd test was performed by serotesting all cattle older than two years and those between birth and six months of age. Routine testing of all herds was halted in 1982 as well as was the policy of test and removal. After that, systematic random testing of sera was carried on at slaughterhouses. In 1985, 0.006% of 149300 cattle were reported as infected with BLV.

A voluntary control programme similar to the Danish programme was established in the Federal Republic of Germany in 1964 and became an official programme in 1973. In 1983, fewer than 0.50% of all herds and 0.05% of all cattle tested were infected with BLV. The number of cattle with EBL decreased from 1548 in 1968 to 114 in 1986 whereas there were 111 infected herds in 1987. Eradication is expected to achieve by the year 2000 (Kautzsch *et al.,* 1990).

Many other European countries have established BLV eradication programmes since the 1980s. Prior to 1982, when the haematological technique was used for screening, the feasibility of eradication was poor. At the present time, Denmark, the Netherlands and Switzerland have been declared BLV free (Radostits *et al.,* 1994), (Stark 1996).

In Australia a government-supported voluntary accreditation scheme was introduced in late 1983 in Queensland and control programmes have been implemented in other states. New Zealand has embarked on a control and eradication programme against BLV since 1996. The objectives of this scheme are to screen, identify and remove BLV-infected animals from every dairy herd in the country as well as achieving internationally recognized status of industry freedom of the disease. The target is aimed to be accomplished by 2004. The following section of this review will outlined the key features of the New Zealand BLV control and eradication scheme.

The New Zealand Enzootic Bovine Leukosis eradication scheme

Since 1996, the New Zealand dairy industry has embarked on a program to eradicate Enzootic Bovine Leukosis and Bovine Leukaemia Virus infection from the national dairy herd. The main reasons for developing an eradication program are as follows:

1. The low prevalence of BLV infection in New Zealand (Hayes 1998) provides an opportunity to eradicate the disease quickly, easily, and cheaply with minimal disruption to individual herds.
2. Potential economic losses caused by BLV infection on-farm can be eliminated. BLV infection will continue to spread if a control scheme is not introduced.
3. International recognition of New Zealand dairy herds being BLV free is expected to provide long-term marketing advantages for product and animal exports.

The EBL Control Scheme has been initiated and funded by the New Zealand Dairy Board for the New Zealand dairy industry. Key bodies involved in the activities of the program are: MAF Quality Management, the Livestock Improvement Corporation, the National Milk Analysis Centre and private veterinary practitioners.

The EBL Control Scheme is made up of two parts:

1. Herd and animal testing to identify sources of BLV infection.
2. The recommendation of farm management practices to minimise herd infection and spread of BLV within the herd.

A flow diagram of the testing procedure to be used on all dairy herds is shown in Figure 2-9. All dairy herds (those herd testing and non-herd testing) are screened for the presence of BLV using samples taken from bulk milk supplied to dairy companies. This process serves to identify those herds containing BLV-positive animals. The diagnostic technique for this stage requires a test of high sensitivity and for the New Zealand EBL Control scheme the biwell ELISA test kit (Rhône Mérieux) is used.

All herd testing herds with a negative bulk milk test are scheduled for pooled milk test at the time of herd recording. Those that return a positive bulk milk test are scheduled for an individual milk test at the next herd recording. Non-herd testing

herds with a positive bulk milk test are scheduled for individual cow milk testing which is carried out by MAF Quality Management technicians.

Pooled milk testing involves "pooling" herd test samples from groups of 20 cows within the herd. This process avoids the cost of sampling individual animals while maintaining the ability to detect the presence of BLV within the herd. For all herd testing herds a negative pooled milk test will result in the herd being declared provisionally BLV free. A positive pooled test results in a herd being scheduled for individual milk testing. Pooled milk samples are tested using the monowell and biwell ELISA technique.

Individual milk testing is the third stage of the testing procedure and is used to identify individual animals within the herd that are suspected of being BLV-positive. Those animals that return a positive individual milk test are scheduled for confirmatory blood testing. Individual milk samples are tested using the biwell ELISA technique and blood samples are tested using both monowell and biwell ELISA test.

The cycle of bulk milk sampling, pooled milk sampling and individual milk sampling is repeated annually. Those herds achieving negative tests for three consecutive years are declared EBL free.

For the first three years of BLV testing, a threshold number of positive individual milk and blood tests in the milking herd determine which *other* classes of stock on-farm are required for blood sample collection and testing. The purpose of these thresholds is to distinguish between: (1) low prevalence herds where only one or two BLV-positive animals are present and no new infections are occurring, and (2) high prevalence herds where a number of BLV-positive animals are present and new infections are occurring. The individual milk and blood test thresholds for testing other stock on-farm in the first three years are shown in Table 2-6.

Recommendations to minimise herd infection and spread of BLV within the herd

Once BLV-positive animals have been identified, herd managers are encouraged to work with their local veterinary practitioner to develop strategies to prevent further introduction of BLV into the herd and reduce spread of BLV within the herd.

The EBL Control Scheme handbook (The New Zealand Dairy Industry 1997) recommends special attention to the following areas:

1. Clear identification of BLV-positive animals: animals identified as BLV-positive are required to have special ear tags applied to identify them as such.
2. Animals should be dehorned using procedures that cause minimal bleeding. If this is not possible strict hygiene levels should be applied at the time of dehorning. Animals should be dispersed into a paddock immediately after dehorning to reduce the potential for contact.
3. Parenteral medication should avoid using common needles and syringes. In particular, known infected animals should be treated separately. In the case of herd vaccination, automated vaccination guns are acceptable provided that needles are replaced at regular intervals.
4. In the case of rectal examinations, gloves should be changed if bleeding occurs. Known infected animals should always be tested separately.
5. Feeding of colostrum to calves should be done only for the first 24 to 36 hours. Pooled calf milk should be obtained from animals within the herd which have been tested negative to BLV infection.
6. Culling of BLV-positive animals should be carried out as early as practical.
7. Testing of animals prior to introduction into the herd is considered as the most important control measure for preventing EBL infection.

Under the current strategy, the New Zealand dairy industry expects to have eradicated EBL by June 2004.

Table 2-1: Clinical signs associated with Enzootic Bovine Leukosis (Johnson *et al.*, 1991a).

Clinical signs	Group 1[a] (%)	Group 2[b] (%)
Weight loss	NR	80
Agalactia	NR	77
Lymphadenopathy	58	58
Anorexia	62	52
Posterior paresis/paralysis	16	41
Fever	NR	23
Exophthalmos	9	20
Laboured breathing	NR	14
Gastrointestinal obstruction	19	9
Myocardial abnormality	64	7
Abnormal blood lymphocytes	63	NR

NR: not recorded
[a] 298 hospitalised cattle
[b] 1100 field cases

Table 2-2: Summary of the most common diagnostic methods for the detection of BLV infection.

Assay	Principle	Sensitivity	Specificity	Advantages	Disadvantages
AGID	Antibody-based testing	98.5% or 94.6%[1]	99.5% or 96.4%[1]	Detects infected cattle as early as 2-4 weeks after exposure. Rapid, inexpensive and can make use of spoiled sera.	Less sensitive, non-quantitative result. Cannot differentiate between passive immunity and infection, especially in cattle less than 6 months of age. Cannot be used on milk.
RIA	Antibody-based testing	100%[2]	100%[2]	Used when compared with serum samples having low antibody titres. Able to detect infection < 2wks after exposure.	Requires sophisticated equipment, therefore expensive. Not suited for mass-screening purposes.
ELISA	Antibody-based testing	98.5%	99.9%	High sensitivity and specificity. Can detect antibody in both milk and serum samples. Can differentiate between passive immunity and acquired infection in calves. Recommended for EBL control programmes, particularly for surveying herds already declared BLV free.	More expensive and requires more sophisticated equipment than AGID.
PCR	Nucleic acid-based testing	Allows detection of 1 BLV-infected cell in 10^4 lymphocytes		Able to detect infection soon after exposure. Sensitive and specific test.	Expensive and requires sophisticated equipment. Not recommended for a mass screening.

[1] compared with RIA
[2] relative sensitivity and specificity to AGID

Table 2-3: BLV prevalence survey studies conducted in New Zealand 1979 – 1999 (Hayes 1998).

Year	Total number of herds tested	Total number of herds positive	Herd prevalence (%)	Within-herd prevalence	Testing method
1979	6062	3	0.05	NA	Blood
1988	25780	69	0.27	NA	Blood
1989	14188	40	0.28	NA	Blood
1990	11357	10	0.09	1 - 50	Blood
1991	4468	105	2.50	5 - 10	Blood
1997 – 1998	14336	889	6.20	NA	Blood
		113	0.79	NA	Milk - individual
		133	0.93	NA	Milk - pooled
		52	0.36	NA	Milk - pooled
1998 – 1999	374	98	61.3	0.5 - 15.5	Blood
Ongoing		6	33.3	NA	Milk - individual
		17	34.6	NA	Milk - pooled

NA: not available

Table 2-4: Important epidemiological features of BLV infection in cattle (Hopkins *et al.*, 1997).

Characteristic	Features
Geographic distribution	Infection occurs worldwide but is clustered in some areas.
Production type	Dairy cattle have higher prevalence than beef cattle
Breed	Unlikely to be direct susceptibility
Sex	No sex predilection
Age	In utero transmission to foetus and horizontal transmission peak at 0.5-3 years old
Genetic factors	BoLA type influences infection
Herd size	High prevalence tends to occur in bigger herd size
Season	No seasonal pattern

Table 2-5: Relative importance of BLV transmission risk factors.

Risk factor	High risk	Low risk	Transmission rate/ Odds ratio
Vertical transmission			
Infected dam	*		0.5 - 8.0%
Colostrum and milk	*		6.0 - 16%
Ova and semen		*	
Horizontal transmission			
Purchasing stock	*		6.0 - 6.6
Mechanical dehorning without disinfectant	*		
Minor procedures permitting blood transfer	*		
Rectal palpation with common sleeves	*		2.8 (95% CI = 1.1 - 6.8)
Calving cows in herd	*		
Close physical contact	*		2.9
Ear tattooing and ear tagging		*	88 - 90% in experimental sheep
IM, SC, ID injection (including vaccination)		*	
Natural breeding, AI, embryo transfer		*	0%
Haematophagous insects		*	
Cross species infection		*	0%

IM: intramuscular; SC: subcutaneous; ID: intradermal; AI: artificial insemination

Table 2-6: Thresholds for testing other stock on-farm in the first three years.

Year of control programme	Individual milk thresholds	Individual blood thresholds
Year 1	≥ 8 positive test	≥ 5 positive test
Year 2	≥ 4 positive test	≥ 2 positive test
Year 3	≥ 1 positive test	≥ 1 positive test

Figure 2-1: Bovine Leukaemia Virus (arrow) in a thin section of infected ovine spleen cells. Bar represents 200 nm (Parrish *et al.*, 1982).

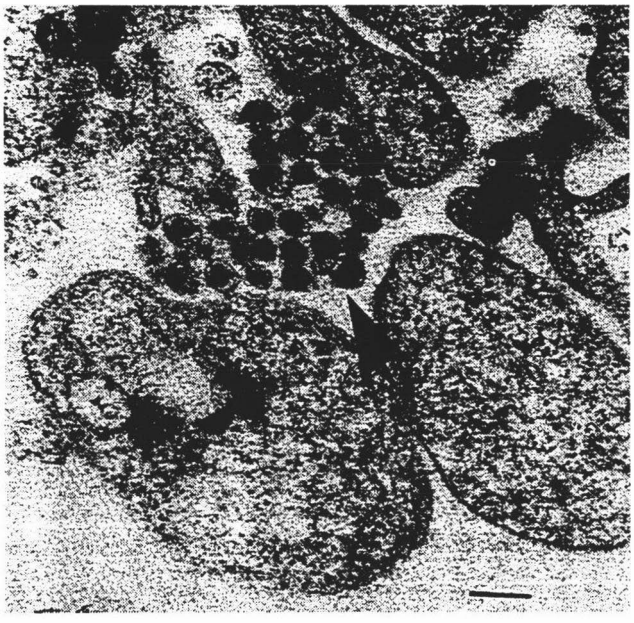


Figure 2-2: Diagram of the structure of the Bovine Leukaemia Virus (Anon. 1999b).

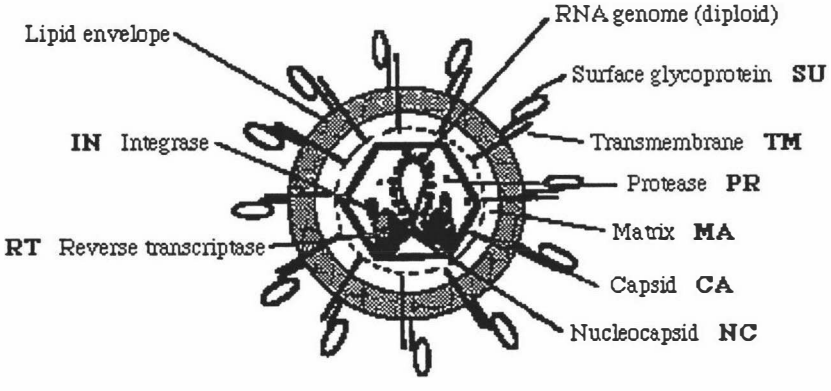


Figure 2-3: Expression of BLV-coded genes, BLV-coded proteins, the host immune response, and the preclinical and clinical phases of BLV infection (Evermann 1992a).

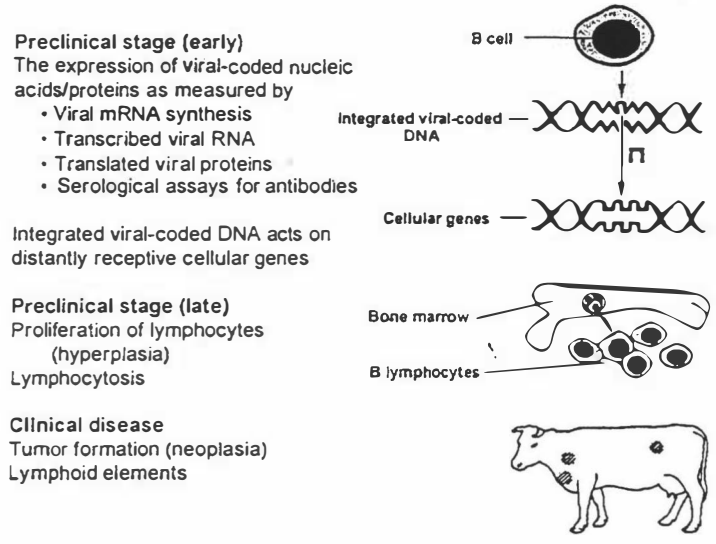


Figure 2-4: Proposed stages of Bovine Leukaemia Virus infecion leading to clinical disease (modified from Evermann and DiGiacomo, 1987).

Viral **transmission** →	Viral Infection →	Precancerous state →	Clinical disease
1. **Vertical** → Epigenetic BLV infection occurs from dam to calf *in utero* (transplacental infection)	Detected by development of antibodies to BLV normally 0.5% - 8%	Aleukemic (normal lymphocyte counts) ↓ ↑ Persistent lymphocytosis (elevated lymphocyte counts)	Lymphosarcoma or lymphocytic leukemia
2. **Horizontal** → cell-associated spread of BLV — infected lymphocytes, e.g. blood-contaminated instruments, insects, etc.	3 to 4 months	30-33%	0.1-5%

Figure 2-5: Worldwide distribution of BLV infection(OIE, Handistatus, 1997).

Figure 2-6: Regional BLV prevalence in New Zealand (Hayes 1998).

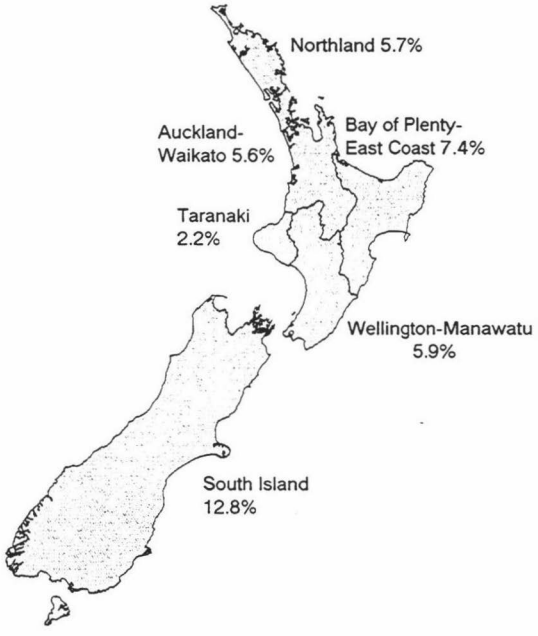


Figure 2-7: Herd and animal prevalence of BLV infection in dairy herds surveyed by NAHMS in 1997 (Anon 1998).

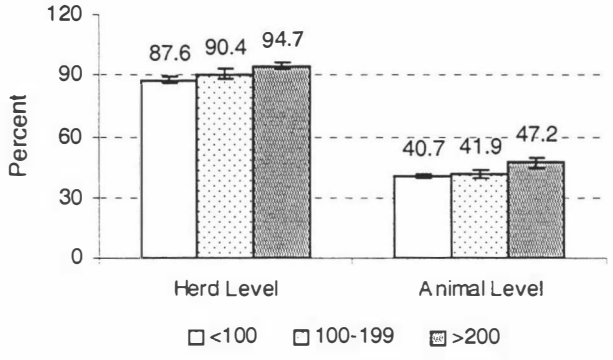


Figure 2-8: Pre and postpartum interrelationships of BLV antibody in the dam, foetus and new-born calf (Johnson *et al.*, 1991a).

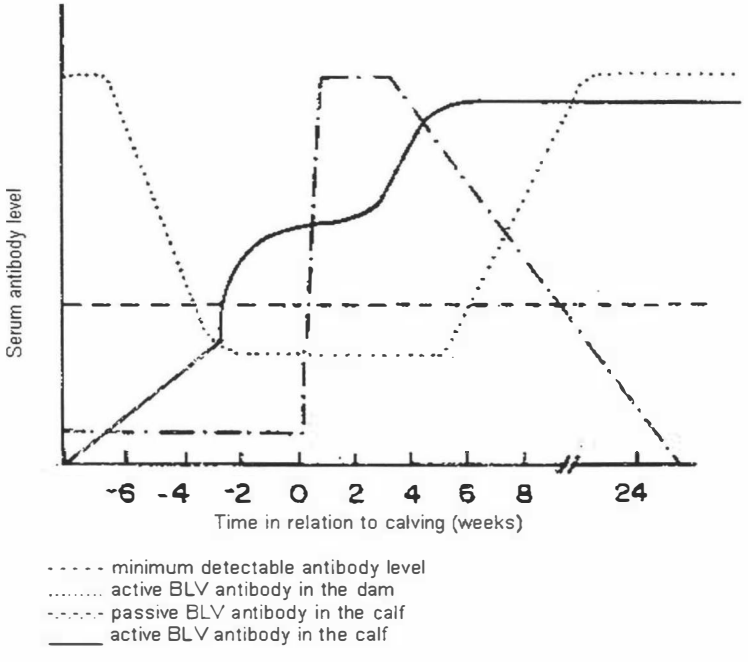
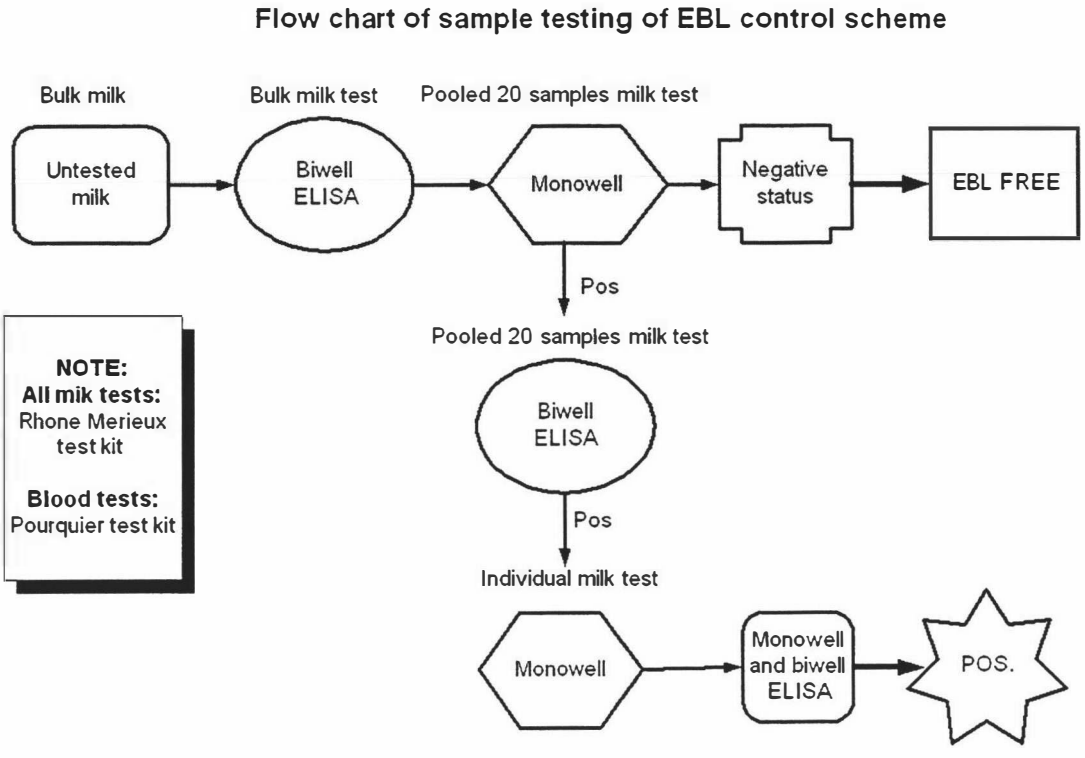


Figure 2-9: A flow diagram of EBL testing procedures.



CHAPTER 3 -

Univariate description of herd-associated risk factors for Enzootic Bovine Leukosis in New Zealand

Introduction

Bovine Leukaemia Virus (BLV) is a viral infection of cattle caused by a type-C oncovirus of the retroviridae family. Although the majority of infections lead to a lifelong antibody response approximately 0.5 - 5.0% of infected animals (Radostits *et al.,* 1994) develop a malignant lymphosarcoma (Enzootic Bovine Leukosis, EBL). BLV infection occurs when infected lymphocytes are transferred from one animal to another and many management practices have been shown to transmit infection. Procedures associated with transmission include gouge dehorning with a common instrument, injection with a common needle and surgical procedures that permit the transfer of blood. Rectal palpation with a common sleeve is also reported to transmit infection (Hopkins *et al.,* 1997).

Recent surveys in New Zealand dairy herds indicate that approximately 6.5% of dairy herds have bulk milk samples that show evidence of BLV activity within the herd (Burton *et al.,* 1997). Prevalence of infected herds ranges from 2.5 to 10% in different regions of the country. Within-herd prevalence of BLV infection, for twenty-five positive herds, ranged from 0.2 to 25%. Given the low prevalence of infection throughout the country, an industry-managed program was started in 1996 to eradicate the disease from the national dairy herd. Under this scheme, dairy farmers have bulk milk tested for BLV antibodies. If bulk milk testing identifies that a herd contains BLV-positive animals, individual milk and/or blood sampling is undertaken. Once BLV-positive animals are identified, herd managers are able to manage these animals appropriately in order to reduce within-herd spread of infection and ultimately eradicate the disease.

While guidelines for reducing between-herd and within-herd spread of BLV infection are well established, the relative importance of the various risks for spread of infection between and within herds are largely unknown (Hilbink *et al.,* 1993).

The aim of the current study was to use the results of the 1996 round of bulk milk testing of the New Zealand EBL eradication scheme to identify BLV-positive and BLV-negative dairy herds. A questionnaire sent to herd managers sought to identify factors associated with the presence or absence of BLV infection in a herd, and putatively with the maintenance of an infected state by within-herd spread of infection. As a result of the comprehensive nature of the questionnaire used in this study the data presented provides detailed descriptive information of the management practices currently used in dairy farms in New Zealand.

Materials and methods

Data collection

This was a case control study using data collected from a mailed questionnaire. Dairy herds selected as cases were those which had either a positive milk vat or pooled animal BLV ELISA test during the 1996 round of testing carried out by the New Zealand EBL eradication scheme. Controls were randomly chosen from herds which had negative result to the same round of testing. Herds with inconclusive results from this round of testing were excluded from selection.

In total 1115 herds were selected to participate in the study with 601 designated as cases and 514 as controls. Information on each herd was obtained by questionnaire administered by mail to the herd manager (see Appendix A). Prior to starting the study the questionnaire was pre-tested with relevant specialists and on five people with dairy herd management experience. Herd managers who did not return their completed questionnaires within 6 weeks were followed up by telephone to improve the response rate and questionable answers were checked by phone. The recall period was up to 24 months prior to the date of completing the questionnaire.

Data processing and analysis

Data were stored in a relational database (Microsoft Access version 7.0, Microsoft, Redmond USA). After transferring questionnaire responses into the study database a 5% random sample of questionnaires was checked against the database to ensure that coding and data entry errors were at an acceptably low level. Data quality was checked by screening the database for out-of-range or missing observations.

The essence of this analysis was to make a comparison of questionnaire information provided by case and control herds in order to identify factors which either increased or decreased the risk of the herd having a positive milk vat or pooled animal BLV ELISA test during the 1996 round of EBL testing. Descriptive statistics of each of the variables recorded in the questionnaire were conducted using SPSS version 8.0 (SPSS Inc., Chicago, USA). Differences between cases and controls for continuous data were assessed using the Student's *t* and the Mann-Whitney U test. For dichotomous data differences between cases and controls were tested using the Pearson's χ^2 test without correction for continuity. For categorical variables odds ratios with 95% confidence intervals were calculated using Epi Info version 6.0 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA). Exact P values were calculated for differences between groups for dichotomous data and Fisher's exact test was used in cases where there were cell sizes of less than five.

Results

Response rate

Of 1115 questionnaires mailed there were 719 replies yielding a response rate of 65%. This sample size represented 5% of dairy herds registered on the Livestock Improvement Corporation database in 1996. The total number of adult dairy cows covered by the questionnaire was approximately 168000. In total 286 herds classified as cases and 433 herds were classified as controls.

The survey sought information in four main areas: (1) farm-specific details, (2) herd demographic details, (3) stock management details, and (4) details concerning the personal characteristics of the interviewee.

Farm-specific details

Details of farm-specific information are shown in Tables 3-1 and 3-2. The majority of case and control herds were seasonally calving with 91% of all herds calving in the spring, 1 - 2% calving in the autumn and the remainder reporting either a bi-seasonal or non-seasonal pattern. Case herds were larger (Mann-Whitney U test statistic 45766; $P < 0.01$) and had been established for a shorted period of time (Mann-Whitney U test statistic 39994; $P < 0.01$) compared to control herds. Average

bulk milk somatic cell count for both groups was low (median 150000 cells.mL^{-1}) and did not differ significantly between groups (Mann-Whitney U test statistic 53268; P = 0.03).

With respect to stated health problems that were of concern to the interviewee, metabolic and mineral deficiencies, calving-associated disorders, reproductive inefficiency and udder disorders were commonly listed. Among these, udder diseases were the most common, reported by 85% of both case and control groups.

Herd demographic details

Details of herd demography are shown in Tables 3-3 and 3-4. Half of the respondent farms produced their own replacement stock. Fifty-seven percent of case herds and 36% of control herds bought adult cows (Table 3-4) whereas 38% of cases and 22% of controls bought heifers for replacement purposes. These animals were purchased from 2 to 4 farms on average and were commonly non-pedigree animals. Of the pedigree animals that were purchased, the most common breed was Friesian. Among farmers who bought stock, measures stated to avoid the introduction of disease were: (1) checking the animal's history with the vendor, (2) applying preventive treatments at the time of purchase (vaccination, worming and quarantine) and (3) monitoring disease and production after purchase. Despite these intended disease control measures, the purchase of adult and replacement heifer stock was associated with a significantly higher risk of the herd being BLV positive (OR 2.4 95% CI 1.7 - 3.2 and OR 2.2 95% CI 1.5 - 3.0, respectively) (Table 3-4).

Grazing practices, providing the potential for animals from one herd to mix with animals from another, were associated with a higher risk of being BLV positive. Those herds that grazed stock from other farms on their property were at 1.6 (95% CI 1.2 - 2.2) times more likely to be BLV positive, compared to control herds.

Ninety percent of case herds and 82% of control herds obtained bulls from an external source. Herds that purchased bulls from an external source were 1.9 (95% CI 1.2 - 3.2) times more likely to be BLV positive. Only 14% of cases and 22% of controls knew whether purchased bulls had or had not been used in other herds prior to purchase.

Stock management details

Details of stock management are shown in Tables 3-5 and 3-6. Calves were left with their dams for a short period after birth (between 12 and 24 hours) after which time they were reared individually or in groups on either whole milk or milk replacer. The type of calf rearing facility had no clearly recognisable association with herd BLV status (Table 3-6), whereas the regular use of discarded milk for calf feeding (colostrum, mastitic, or witheld milk) was significantly associated with the risk of the herd being BLV positive (OR 2.6 95% CI 1.0 - 7.2).

Eighty-eight percent of case herds and 75% of control herds reported that either some or all of the adult herd was pregnancy tested. Pregnancy testing the entire adult herd was associated with a 2.4 (95% CI 1.6 - 3.8) times risk of being BLV positive.

Use of Clostridial vaccination was reported by 78% of cases and 85% of controls. Herds using Clostridial vaccination were 0.7 (95% CI 0.4 - 1.0) times likely to be BLV positive. Dehorning, was related to herd BLV status. Dehorning calves at greater than 4 weeks of age increased the risk of the herd being BLV positive, but not significantly so (OR 1.4 95% CI 1.0 - 2.0). Stating that no methods were used to prevent the spread of disease at the time of dehorning increased the risk of the herd being BLV positive by 2.7 (95% CI 1.6 - 4.6).

Characteristics of the interviewee

Personal details of the survey respondents are shown in Table 3-7. Most of the interviewees were owners with the remainder of respondents describing themselves as sharemilkers. Fifteen percent of all respondents had received education higher than that of on-farm training. Interviewee characteristics that increased the risk of the herd being BLV positive were: (1) having some formal level of qualification (OR 1.5 95% CI 1.0 - 2.2), (2) being a sharemilker (OR 1.6 95% CI 1.1 - 2.3), (3) being between 25 - 39 years of age (OR 2.9 95% CI 2.0 - 4.0) and (4) stating that a farm advisor was used to assist with herd decision making (OR 1.4 95% CI 1.0 - 1.9).

Discussion

A case control study is a rapid and inexpensive method to investigate risk factors for disease occurrence but relies on accurate definition of herds into cases and controls.

In this study, allocation of study subjects into case and control categories was objective with the results of bulk milk tank ELISA testing used to determine herd infection status. Respondents to the questionnaire had not received the results of bulk milk testing, so there was no chance of knowledge bias influencing their responses. An advantage of a questionnaire covering a broad range of factors associated with dairy management is that a management "profile" of a BLV positive herd can be defined. Once veterinarians are able recognise "at risk" management profiles, in the absence of serological data to confirm the herd's true BLV status, particular care can be taken to reduce possible transfer of infection.

Since the outcome of interest in this study is herd BLV status, this survey has sought to identify those factors that might influence the spread of BLV from one herd to another. Additionally, the effect of factors typically associated with within-herd spread of BLV were investigated (e.g. is gouge dehorning used, is pooled colostrum used) and assessed for their influence on herd BLV status. With respect to the interpretation of the influence of this second category of factors, the implication of a positive association between a risk factor and herd BLV status is that the presence of the factor has enabled BLV infection to persist within the herd, and therefore rendered it positive to bulk milk tank testing.

Farm-specific details

Case farms had larger herds than controls and herds of over 200 cows were more than 2.3 times more likely to be BLV positive compared to herds of less than 200 cows. Recently established herds were at higher risk of being BLV positive than longer-established herds, with risk declining progressively with years since establishment. These findings suggest that newly-established, larger herds (having a tendency to be made up of stock purchased from multiple sources) are more likely to be BLV positive compared to longer established, smaller (and often closed) herds.

Disease control practices

Most respondents stated that measures were taken to avoid the introduction of disease into their herd. Case herds were more likely to practise a number of actions that inadvertently exposed them to introducing BLV, such as buying stock from outside the area, and mixing their cattle with stock from other farms. Purchasing

stock was positively assoicated with being BLV positive (OR 2.2 - 2.4 95% CI 1.5 - 3.2). This finding is in agreement with studies in Spain (Casal *et al.,* 1990), Australia (Dimmock *et al.,* 1991) and Canada (Sargeant *et al.,* 1997b). Casal *et al.* (1990) and Thomas *et al.* (1984) found that the appearance of new BLV foci was almost always a consequence of purchasing practices.

The rearing of replacement stock away from the main farm area was a significant risk factor for being a BLV positive herd (OR 1.7). Stock grazed away from the main farm area are presumably at risk of being exposed to BLV as a result of physical contact with animals from other herds. Similarly, obtaining bulls from external sources was a risk factor for being BLV positive (OR 1.9 95% CI 1.2 - 3.2). Where the respondent had little knowledge of the origin of bulls obtained, or was indisciminant in sourcing bulls, the risk of the herd being BLV positive was higher (OR 3.2 95% CI 1.8 - 5.8).

Stock management

Confinement of groups of cows during the calving period is a management factor that carries the potential for blood, tissues and uterine fluids to be transferred from one animal to another. As a consequence, calving in a specially isolated springing (i.e. late pregnant) group, where pregnant cows are confined closely, was a risk factor (OR 1.6 95% CI 1.1 - 2.2) for herds being BLV-positive.

Husbandry techniques associated with calf rearing were found to be related to the presence of BLV infection to some extent in this study, although the findings were not consistent. Both horizontal and vertical transmission of BLV infection may occur during calf rearing depending on the number of infected lymphocytes present in the blood, colostrum and milk of the dam (DiGiacomo 1992b). Leaving the calf with the cow for more than 24 hours was protective, but was not commonly practised. Type of calf rearing facility had a small effect, but the pattern was not readily interpretable in epidemiological terms, and may have resulted principally from confounding with other practices.

Among the types of discarded milk, feeding pooled colostrum had the strongest association with presence of BLV infection (OR = 2.6 95% CI 1.0 - 7.2). Given the high cellular content of colostrum, this is finding is biologically plausible. Milk-borne infection has previously been reported in calves, with 6 to 16% of infection

being attributed to this cause by 6 to 12 months of age (DiGiacomo 1992b) . In fact, the susceptibility of calves to infection by colostrum or milk was exacerbated by the absence of colostral antibodies to BLV and when the calf's age at the time of feeding was less than 4 days (Hopkins *et al.,* 1997). Calves with no detectable antibodies during the first week of life were found to be 2 - 3 times at risk compared with those which had antibodies (Lassauzet *et al.,* 1989a). Evidence indicated that calfhood infection may be reduced by about 45% through the feeding of colostrum with BLV antibodies and further feeding with other milk-modified products (Lassauzet *et al.,* 1989a).

Herds that pregnancy tested were more than twice as likely to be BLV-positive compared to those which did not, although it is likely that this finding is confounded in this analysis with herd size and management style. The time when pregnancy diagnosis was conducted was associated with BLV status. In particular, pregnancy testing at the end of the season was strongly associated with a positive BLV status (OR 3.6 95% CI 2.2 - 5.8).

Iatrogenic transmission by routes such as vaccination, injection and drenching was not identified in this study, and use of these practices was found not to be associated with a herd being BLV positive. Dehorning using methods which would enhance transfer of blood between animals showed marginal association with presence of infection, the strongest association being when no disease prevention methods were used in association with dehorning. An observational study on the field use of a blood contaminated dehorning device without cleaning or disinfection resulted in seroconversion three months after dehorning (DiGiacomo *et al.,* 1985). The relative risk calculated from the prevalence in that study was 3.5 fold (P = 0.01) whereas calculation from the incidence was 5 fold (P = 0.2).

Herd owner-specific details

It was assumed in this study that the person responding to the questionnaire was responsible for management of the herd. BLV positive herds were more likely to have a younger person managing the herd, who was a sharemilker and had some form of training other than on-farm experience. All of these effects except age were marginal, and probably due to newer farmers buying animals to establish a (larger than average) herd, and therefore introducing BLV infection into the herd.

Conclusion

This study has identified at the univariate level demographic and management factors that are associated with the presence of BLV infection in dairy herds in New Zealand. The findings identified here are broadly consistent with overseas research. Clusters of variables in this study are likely to be highly correlated, and therefore some are unlikely to be truly causal. Multivariable analysis of the data will help differentiate more clearly those which are potentially causal from those which are contributory but not causal, and those which are simply confounded with a causal or contributory factor.

Table 3-1: Descriptive statistics for continuous variables associated with farm-specific details and results of Mann-Whitney U test for differences between BLV positive and BLV negative herds.

Outcome	Cases				Controls				test statistic	P
	n	Q1	Q2	Q3	n	Q1	Q2	Q3		
Number of milking cows	282	169	230	320	433	145	187	245	45776	< 0.01
Number of years at current location	280	5	15	30	421	14	15	24	39994	< 0.01
Mean somatic cell count for season (x 1000 cells/mL)	276	120	150	200	428	105	150	180	53268	0.03

Q1: 25th quartile

Q2: 50th quartile

Q3: 75th quartile

Table 3-2: Counts of categorical variables associated with farm-specific details and unadjusted odds ratios indicating the risk of a herd being BLV positive, given specified exposure.

Outcome	Cases		Controls		OR	95 %CI
	No. herds	%	No. herds	%		
Maximum number of milking cows:						
> 200	175	62	182	42	2.3	1.6 - 3.1
Number of years herd has been established: [a]						
≤ 3 years	96	34	81	19	4.3	2.6 - 7.1
4 - 18 years	123	44	154	37	2.9	1.9 - 4.6
19 - 25 years	24	9	51	12	1.7	0.9 - 3.3
Person responsible for management of herd: [b]						
Sharemilker	112	39	124	29	1.7	1.2 - 2.4
Herd health disorders perceived to be important by the interviewee: [c]						
Metabolic diseases or mineral deficiency	114	43	225	56	0.6	0.4 - 0.8
Stress	45	17	121	30	0.5	0.3 - 0.7

[a] reference category: herds established > 25 years
[b] reference category: owner of herd
[c] reference category: no predominant health disorders perceived to be present

Table 3-3: Descriptive statistics for continuous variables associated with herd demographic information and results of Mann-Whitney U test for differences between BLV positive and BLV negative herds.

Outcome	Cases				Controls				test statistic	P
	n	Q1	Q2	Q3	n	Q1	Q2	Q3		
Percent of stock sent to slaughter	281	0	9	17	428	3	13	19	53818	0.02
Percent of stock sent to other than slaughter	274	0	0	7	424	0	0	11	51500	0.01
Number of weeks artificial breeding used during breeding season	278	4	5	6	430	5	5	6	56759	0.24
Number of weeks herd naturally mated after artificial breeding ceased	275	6	7	9	411	6	7	8	56491	0.72

Q1: 25th quartile; Q2: 50th quartile; Q3: 75th quartile

Table 3-4: Counts of categorical variables associated with herd demography and unadjusted odds ratios indicating risk of a herd being BLV positive, given specified exposure.

Outcome	Cases		Controls		OR	95% CI
	No. herds	%	No. herds	%		
Purchasing stock: [a]						
Adult stock purchased	159	57	155	36	2.4	1.7 - 3.2
Replacement heifers purchased	104	38	94	22	2.2	1.5 - 3.0
Sources of purchasing stock class: [a]						
Purchased from local region	144	54	165	37	2.4	1.7 - 3.4
Purchased from other region	60	21	46	11	3.4	2.1 - 5.6
Measures to avoid the introduction of disease: [b]						
Animal history, certification and inspection	27	11	47	12	1.8	1.0 - 3.2
Disease prevention: [c]						
Preventive measures stated	130	51	125	31	1.6	1.0 - 2.5
Pedigree stock: [d]						
Pedigree stock present	153	55	181	42	1.7	1.2 - 2.3
Friesian pedigree stock present	120	43	109	26	2.1	1.5 - 3.0
Grazing: [e]						
Other farmers' cattle grazed on farm	73	26	76	18	1.6	1.2 - 2.2
Stock grazed off farm	181	64	224	52	1.7	1.2 - 2.3
Heifers grazed off farm and don't mix with other stock	157	57	186	44	1.9	1.2 - 3.2
Source of bulls: [f]						
Bulls obtained from external source	251	90	353	82	1.9	1.2 - 3.2
Bulls obtained from dairy breeders	43	15	57	13	2.1	1.1 - 4.1
Bulls obtained from beef breeders	154	55	218	51	2.0	1.2 - 3.4
Bulls obtained from unknown sources	80	29	71	17	3.2	1.8 - 5.8

[a] reference category: don't purchase stock
[b] reference category: no measures taken to reduce the introduction of disease
[c] reference category: preventive measures to avoid spread of disease within the herd stated
[d] reference category: pedigree stock not present on the farm
[e] reference category: stock not grazed off the farm
[f] reference category: bull not obtained from external sources

Table 3-5: Descriptive statistics for continuous variables associated with stock management and results of Mann-Whitney U test for differences between BLV positive and BLV negative herds.

Outcome	Cases				Controls				test statistic	P
	n	Q1	Q2	Q3	n	Q1	Q2	Q3		
Age of calves at weaning (weeks)	271	8	9	10	411	8	9	10	-1.010 [a]	0.31
Weight of calves at weaning (kg)	3	50	90	90	17	58	90	100	21.0	0.69
Number of times vet visited farm throughout season	253	2	4	6	332	3	4	5	40240	0.38

[a] independent samples *t* test
Q1: 25th quartile
Q2: 50th quartile
Q3: 75th quartile

Table 3-6: Counts of categorical variables associated with stock management practices and unadjusted odds ratios indicating the risk of a herd being BLV positive, given specified exposure.

Exposure factors	Cases		Controls		OR	95% CI
	No. herds	%	No. herds	%		
Time calf spent with dam: [a]						
More than 24 hours	13	5	44	10	0.4	0.2 - 0.8
Calf rearing facility: [b]						
Reared in shed with no separation	27	10	68	16	0.6	0.3 - 0.9
Reared in separate pens in shed	250	88	355	82	1.7	1.1 - 2.6
Reared outside with shelter	60	21	132	30	0.6	0.4 - 0.9
Type of feeding for calves: [c]						
Feed colostrum	277	98	410	95	2.6	1.0 - 7.2
Feed mastitis milk	159	56	194	45	1.6	1.2 - 2.2
Feed withheld milk	202	71	251	58	1.8	1.3 - 2.5
Place of calving: [d]						
Allocated calving paddock	85	30	167	39	0.7	0.5 - 1.0
In springing group	219	78	299	69	1.6	1.1 - 2.2
Pregnancy testing of the adult herd: [e]						
Pregnancy testing carried out	250	88	325	75	2.4	1.6 - 3.8
Use of ultrasound testing: [e]						
Ultrasound testing used	59	21	71	17	2.6	1.5 - 4.6
Person who pregnancy tests cows: [e]						
Veterinarian	216	76	285	66	2.4	1.5 - 3.8
Contractor	32	11	52	12	2.0	1.0-3.7
Farmer	218	77	273	63	2.5	1.6 - 4.0
Stage of pregnancy testing: [e]						
Early season	51	18	85	20	1.9	1.1 - 3.3
End of season	119	42	106	24	3.6	2.2 - 5.8
Other times	94	33	133	31	2.3	1.4 - 3.7
Treatments: [f]						

Exposure factors	Cases		Controls		OR	95% CI
	No. herds	%	No. herds	%		
Clostridial vaccines	224	78	365	85	0.7	0.4 - 1.0
IBR vaccine	7	3	39	9	0.3	0.1 - 0.6
Parasiticides	9	3	28	6	0.5	0.2 - 1.0
Calving induction	233	83	327	77	1.5	1.0 - 2.2
Supplementary feed: [g]						
Concentrate feeds used	31	11	87	20	0.5	0.3 - 0.8
Dehorning: [h]						
Done at less than 4 weeks of age	115	40	203	47	0.8	0.6 - 1.1
Done at greater than 4 weeks of age	151	53	200	46	1.3	1.0 - 1.8
Mechanical dehorner used	83	29	100	23	1.4	1.0 - 2.0
Preventive measures taken at dehorning: [i]						
Disinfection used	48	17	74	17	0.6	0.4 - 1.0
Unspecified preventive measures used	70	75	45	90	0.4	0.1 - 1.1

[a] reference category: calf left with cow for less than 12 hours
[b] reference category: calves not kept in pens
[c] reference category: calves not fed colostrum
[d] reference category: no special area for calving allocated
[e] reference category: no pregnancy testing undertaken
[f] reference category: no treatment used
[g] reference category: no concentrate feeds used
[h] reference category: dehorning not used
[i] reference category: no hygiene methods applied at time of dehorning

Table 3-7: Counts of categorical variables associated with interviewee characteristics and unadjusted odds ratios indicating the risk of a herd being BLV positive, given specified exposure.

Exposure factors	Cases		Controls		OR	95% CI
	No herds	%	No herds	%		
Status of interviewee: [a]						
Sharemilker	72	25	77	18	1.6	1.1 - 2.3
Age group of interviewee: [b]						
25 - 39 years	144	51	161	37	2.9	2.0 - 4.0
Highest qualification of interviewee: [c]						
Certificate, diploma, or higher qualification	65	24	72	18	1.5	1.0 - 2.2
Use of farm advisor: [d]						
Farm advisor used	189	66	254	59	1.4	1.0 - 1.9
Veterinarian as farm advisor	122	43	168	39	1.3	0.9 - 1.9
Farm management adviser	138	48	189	44	1.3	1.0 - 1.9

[a] reference category: owner
[b] reference category: age group 40 - 54 years
[c] reference category: no formal qualification
[d] reference category: no farm advisor used

Figure 3-1: Histogram showing the distribution of herd size for BLV positive and BLV negative herds.

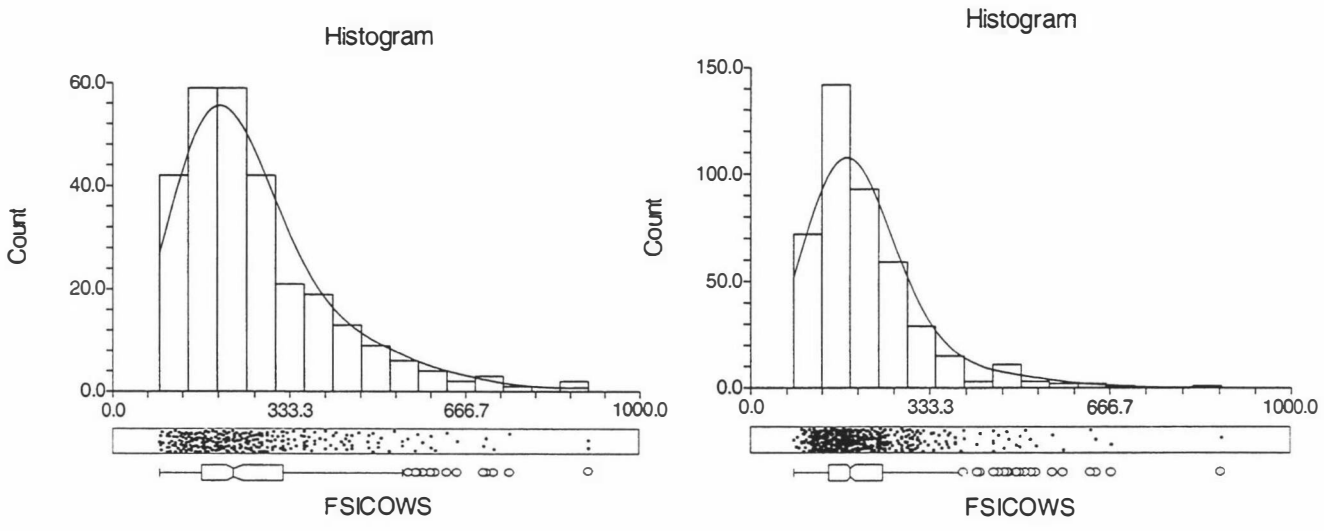


Figure 3-2: Histogram showing the distribution of number of years that herds have been established.

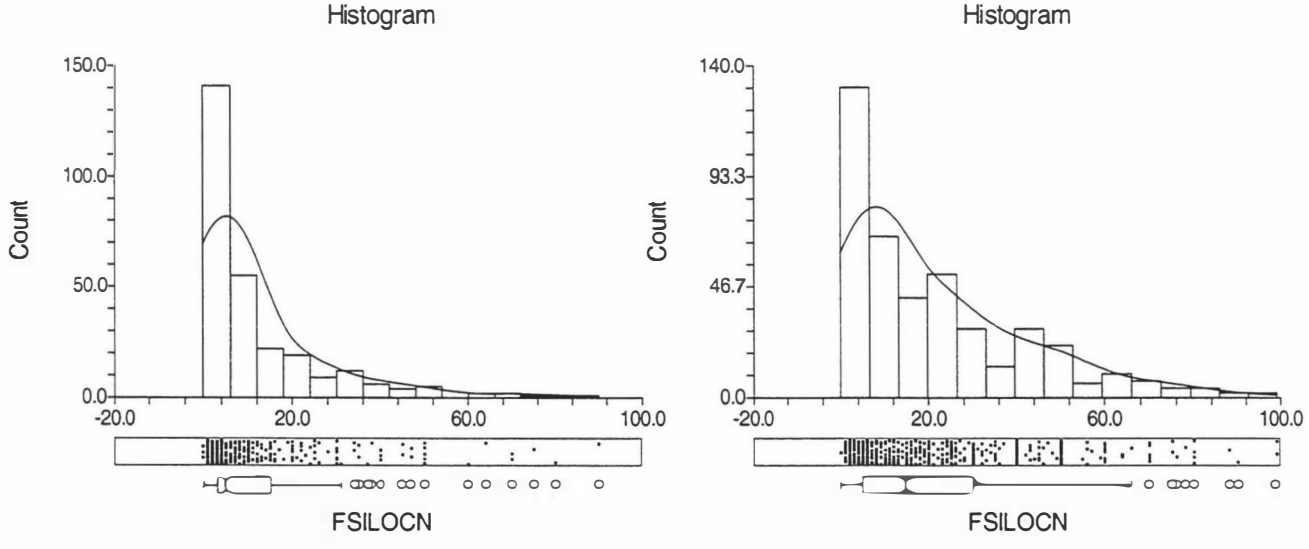


Figure 3-3: Pie chart giving breakdown of the status of those responsible for farm management.

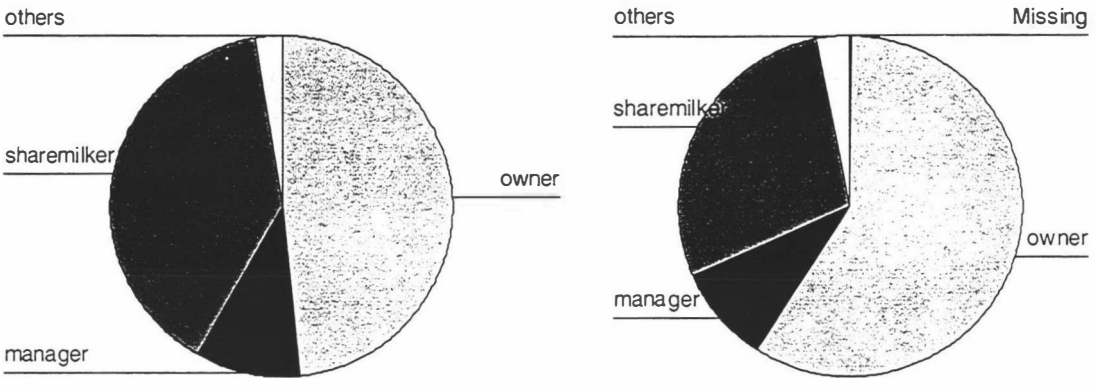


Figure 3-4: Bar chart showing the percentage of herds buying cows from other sources.

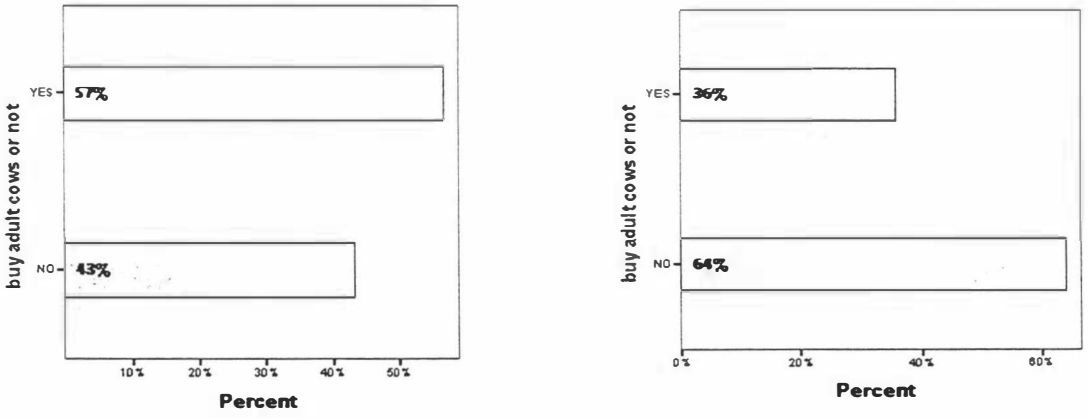
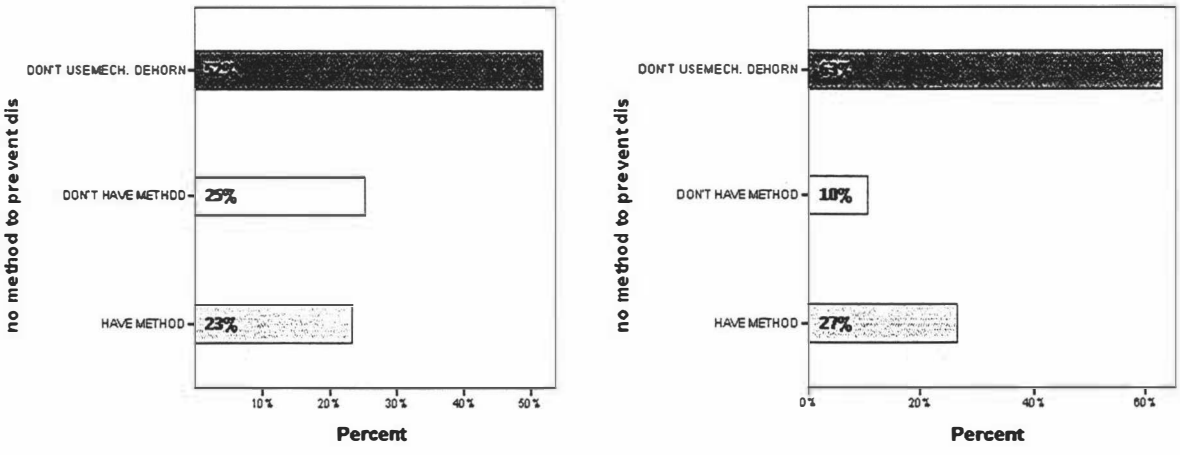


Figure 3-5: Bar chart showing the percentage of herds using mechanical dehorning and not using any measures to prevent disease spread.



CHAPTER 4 -

Multivariable analysis of risk factors for Enzootic Bovine Leukosis in dairy cattle in New Zealand

Introduction

Risk factors of BLV infection in cattle have been widely documented (Hopkins *et al.,* 1997); (Radostits *et al.,* 1994); (Johnson *et al.,* 1991b). Horizontal transmission is considered to be more important than vertical transmission with management practices involving the transfer of blood from one animal to another regarded as the main route of disease transmission. Since the clinical form of BLV infection, Enzootic Bovine Leukosis, is a disease with potential for significant economic impact on the dairy industry, control programs have been implemented in a number of countries. In New Zealand, BLV infection has existed at low prevalence and, consequently, EBL is rarely seen clinically (Burton *et al.,* 1997). Since the presence of BLV infection could potentially become a major trade barrier, an eradication scheme was introduced in New Zealand in 1996.

Univariate analysis of the results of our case-control study indicated that several factors were associated with BLV infection status of a herd (see Chapter 3). However, as with most observational studies, risk factors identified at the univariate level may have confounding or interaction relationships with each other and multivariable analysis techniques are required to further clarify relationships between risks and disease. The objectives of this study were to provide a comprehensive representation of the causal web of risk factors associated with the presence of BLV infection in New Zealand dairy herds using multivariable statistical techniques.

Materials and methods

Study population and data collection

Details of the study design, definition of outcome status and data collected are given in Chapter 3.

Statistical analyses

A range of multivariable analysis techniques were used to describe the underlying causal structure for the outcome of interest in this study, that is whether or not a herd was BLV-positive. In the first instance logistic regression was used to model the relationship between explanatory variables and the dichotomous outcome variable. Secondarily, path analysis was used to produce a representation of the pattern of causal relations among the risk factors identified in Chapter 3. This technique allows the analysis to be influenced by a hypothesised time sequence of the occurrence of risk factors as well as by a biological understanding of relationships within the system. Multiple logistic regression analysis was used to test the statistical significance of direct and indirect effects included in the null hypothesis path model. Thirdly, classification tree analysis was used for to predict the outcome for each herd based on specific risk factor patterns identified for individual observations. This technique is particularly useful to describe factors that identify high risk sub-populations (Heping Zhang *et al.*, 1996). Finally, optimal scaling or dimension reduction was used as a unifying technique in exploratory multidimensional data analysis, linking up with the rationale of classification, regression and clustering. Homogeneity analysis (HOMAL) or multiple correspondence analysis was used as a graphical display technique to identify interdependence among nominally scaled variables and can be thought of as a non-linear principal components analysis (Gifi, A 1990). Unlike conventional statistical methods, homogeneity analysis is an exploratory technique rather than a confirmatory one.

For the multivariable analysis, risk factors measured at a continuous scale were re-coded into categorical variables in order to simplify the interpretation of regression coefficients as odds ratios. These variables, representing 6% of variables in the questionnaire, were categorised based on the median or the quartiles. Variables with more than 5% missing values from this group were excluded. This data set was used in all of the following analyses.

Logistic regression

Since the univariate analysis resulted in a large number of significant variables, separate logistic regression models were developed for specific groupings of risk factors. Each of the models was obtained using stepwise forward selection based on

an entry level of $P < 0.05$ and exclusion at $P > 0.10$ using SPSS for Windows version 8.0. (SPSS Inc., Chicago, Illinois). The final main effects of regression model were checked for significance of potential first-order interaction terms.

Path analysis

A null hypothesis path model was constructed as a diagram representing the most plausible set of possible causal interrelationships of risk factors associated with the presence of BLV infection in dairy herds. The path model was designed as a recursive model, meaning that the causal flow in the model was unidirectional and that there were no feedback relationships between factors. A distinction was made between exogenous and endogenous variables when constructing the null hypothesis path model. An exogenous variable was a variable whose variability was assumed to be determined by causes other than factors represented in the path diagram. The temporal sequence of events is represented in the path model by developing effects from left to right. Thus, any exogenous variables should be presented at the left side of the diagram. An endogenous variable, on the other hand, is one whose variation is explained by exogenous or other endogenous variables included in the model. Paths were drawn with arrows pointing left to right to indicate temporal and putative causal relationships. The arrows were included based on existing knowledge about potential causal relationships between variables. We have included only those paths which we considered biologically plausible or which had been previously documented. Any factors represented in the final logistic model are likely to have direct effects on the presence of BLV infection. The dependent variable herd BLV infection status was included on the right side of the path diagram. The statistical analysis of the path diagram was performed using the logistic regression analysis procedures available in MINITAB (Minitab for Windows, Release 12.1, Minitab, Inc., 3081 Enterprise Drive, State College, PA 16801-3008, USA) and SPSS for Windows version 8.0. The MINITAB software was used when a categorical outcome variable was measured at an ordinal or multi-nominal scale while SPSS was used for standard logistic regression analysis with dichotomous outcome variables. Each variable in the path model was in turn regressed on all the preceding variables that had arrows leading directly to that dependent variable. Insignificant variables or variables resulting in multicollinearity between independent variables were dropped from the null hypothesis path model. Multicollinearity was assessed

on the basis of the presence of regression coefficients with large standard errors, and subsequent analysis of the statistical relationship between predictor variables using correlation or chi-squared analysis. Variable selection was based on a forward stepwise method, with a P-value of 0.05 for entry and 0.10 for removal. The magnitude of the effects was expressed using odds ratios. Statistical significance was indicated in the path diagram through the number of stars attached to a path (* $P < 0.05$, ** $P < 0.01$).

Classification tree analysis

The data set used in this analysis included the same variables as the null hypothesis path model comprising a total of thirty significant variables. The ANSWTREE software version 1.0 (SPSS Inc., Chicago, Illinois) was to perform the classification tree analysis. Of the different tree estimation algorithms in this program, Chi-squared Automatic Interaction Detection (CHAID) was used for this analysis. The stopping rule for all schemes was a maximum tree depth of 5 levels with a minimal parent and child node size of 20 and 10 observations, respectively. Basically, at each step the program determined for each variable a cut point which optimally split the population with respect to discrimination of BLV herd infection status, and then selected the variable which produced the best split. Then, within each of the resulting sub-populations the same method was applied until the stopping rules were met or the subpopulation was completely pure - meaning in one BLV infection status category. The program also provided a risk estimate describing the proportion of observations classified incorrectly and a misclassification matrix counting up the predicted and actual category values. These values were used for calculation of the sensitivity and specificity of the overall tree as well as for individual decision rules. Bias of the resulting tree was assessed using cross-validation. Each population and the pooled population were randomly divided into ten subgroups and each tenth was classified using the risk identification scheme derived from a classification tree analysis of the other nine-tenths. The error rates for the classification of cases and controls were then averaged over all ten analyses.

Multiple correspondence analysis

The same data set used in the stepwise logistic regression was used for a multiple correspondence analysis with the aim to describe latent variable constructs

associated with the BLV herd infection status. A reduced set of indicator variables was selected to describe particular characteristics of farming operations. These included BUYSTOCK, FARMLOCAT, HERDSIZE, FARMERAGE and FARMEDU. The optimal scaling procedure available in the SPSS software, HOMALS, was used to perform a multiple correspondence analysis also known as homogeneity analysis. It scales the variables using a given number of dimensions such that they can be presented graphically, and two dimensions were used in the current analysis. The technique re-scales the data by assigning object scores to each observation, and then estimates for each variable category a category quantification based on the average object scores. The purpose is to identify optimal scores for which the different categories are separated as much as possible. The procedure constructs a map to depict the discrimination levels. The average of the discrimination measures for any dimension equals the eigenvalue (total variance accounted for) for that dimension (Anon 1998). Discrimination measures of variables closely plotted together indicate that these may be associated.

Results

Codes and descriptions of significant variables obtained from the univariate analysis of this data set are shown in Table 4.1.

Logistic regression

The total number of records in this data set was 510, representing 71% of the original data set. The number of cases was 182 and the number of controls was 328. From 80 potential risk factors obtained from univariate analysis, ten factors provided a statistically significant contribution to the presence of BLV infection in the logistic regression analysis. Risks factors for BLV included: number of years of farm establishment (FARMLOCAT), herd size (HERDSIZE), the purchase of stock from external sources (BUYSTOCK), having pedigree Friesian stock (HAVPEDFRI), obtaining non-pedigree bulls off-farm (BULLNPED), not using preventive measures when dehorning (NODISPREV) and performing pregnancy testing at the end of season (PREGEND). Protective factors for BLV were: perceived management stress (MNGSTRESS), use of concentrates as a feed (CONCENTR) and use of Clostridial vaccines (BLACKLEG).

Results of logistic regression are shown in Table 4.2. The goodness of fit index, which measured the relative amount of variance jointly accounted for was 0.536. This model explained 24.8% (Cox & Snell R^2) or 34.1% (Nagelkerke R^2) of the variation associated with the presence of BLV infection in dairy herds.

Path analysis

The null hypothesis path model describing the hypothesised causal web is shown in Figure 4.1, and the final path model in Figure 4.2. Two variables, herd size/ number of milking cows (HERDSIZE) and farmer's age (FARMERAGE) were used as exogenous factors while the remaining variables were endogenous factors. Of 30 variables included in the null hypothesis path model, 10 variables remained in the final path model. Seven of these ten variables were the same as those in the stepwise logistic regression analysis and had direct effects on the presence of BLV infection in herds. They were HERDSIZE, PREGEND, MNGSTRESS, HAVEPEDFRI, BUYSTOCK, FARMLOCAT and NODISPREV. A number of factors (such as the use of Clostridial vaccine) were removed from the path model as a result of insignificant path coefficients. Three factors: farmer's age, farmer education and type of manager had indirect effects on BLV infection status. Table 4.3 shows the odds ratios and 95% confidence intervals for each variable included in the final path model.

There were 505 observations in this analysis representing 70% of the original data set (505/719) or 99% (505/510) of the data set used for multivariable analyses. The number of cases was 181 and number of the controls was 324. The goodness of fit index was 0.500. This model explains 23% (Cox & Snell R^2) or 32% (Nagelkerke R^2) of the variation associated with the presence of BLV infection.

Classification tree analysis

The data set based on variables also included in the null hypothesis path model was used for the classification tree analysis. The resulting tree had 27 nodes stratified into five levels and 14 nodes of which were terminal nodes. Their order was ranked with respect to the strength of association between the risk factors and BLV infection outcome (χ^2). One-third of 30 variables submitted to the analysis were included in the tree diagram ($P < 0.05$). These were herd size, having pedigree

Friesian stock, the purchase of stock from external sources, having management stress, not using preventive measures when mechanically dehorning, performing pregnancy testing at the end of season, use of concentrate as a feed, dehorning animals older than 4 weeks, separating herd by disease and health management and person who did dehorning. The classification tree diagram is shown in Figure 4.3.

Assuming an equal weighting for the costs of false positive and false negative classifications, this tree gave 59% sensitivity and 83% specificity. The overall percentage of misclassification was 26 $\pm$ 1.9% which increased to 37 $\pm$ 2.1% after cross validation.

Multiple correspondence analysis

Graphical output from the multiple correspondence analysis of the BLV data set is shown in Figure 4.4. A number of variables included in the multiple correspondence analysis were closely related to the presence and absence of BLV infection, suggesting that these variables could be used as discriminators between the BLV infection groups. High BLV risk farms were associated with the purchase of stock from external sources, large herd size, new established herds, and farmers holding higher qualifications. The results of category quantifications are shown in Table 4.4. The total inertia was 55%. The first dimension represented 33% of the variance whereas the second dimension accounted for 22%. Of the two dimensions, the first axis was of particular interest because of its strong association with BLV infection status.

Discussion

The univariate analysis of the data from this case-control study resulted in a large number of factors being found to be associated with BLV infection status of dairy herds. The three techniques used in this multivariable analysis were able to consistently identify two major risk factors associated with herd BLV status: herd size (number of milking cows) and the purchase of stock from external sources. Although number of years since farm establishment was not included in the final classification tree, this factor came out as significant in the other analyses. These findings suggest that introduction of animals from external sources, whether as

regular practice for obtaining replacement animals or when setting up a new herd, constitutes a major risk factor for introduction of BLV infection into a herd. If herds are newly established, farmers will have to acquire substantial numbers of animals. If these farmers want to further increase their herd size, they are likely to purchase stock from different sources. This can favour the chance of obtaining infected animals because less discrimination will be practiced during the selection of stock of purchase.

Amongst the other risk factors 'not using preventive measures when mechanically dehorning' and 'performing pregnancy testing at the end of season' are in agreement with findings from several previous studies (Darlington *et al.,* 1984) (DiGiacomo *et al.,* 1990) (Lassauzet *et al.,* 1990) (Henry *et al.,* 1987), and (Divers *et al.,* 1995). These are classified as high risk factors for BLV transmission (Radostits *et al.,* 1994) and (Hopkins *et al.,* 1997). Pregnancy testing and mechanical dehorning involve the potential of blood transfer, which is the dominant mechanism of BLV transmission particularly when no specific measures are taken to prevent disease spread. If pregnancy testing is performed towards the end of the season, it is likely to result in more severe bleeding than when done in the early season. Similarly, dehorning of animals older than 4 weeks can increase the risk of becoming infection as a result of more severe bleeding. The degree of hygiene adopted by the persons performing the dehorning will affect spread of BLV infection. Lastly, the fact that a farmer may separate stock for disease and health management could be interpreted as an indicator of an increased intensity of individual animal health measures which while not being aware of this being a risk factor may involve the use of the same needles for treating multiple animals.

The perceived presence of management stress, the use of Clostridial vaccines and the use of concentrates as a feed significantly reduced the risk of BLV infection in this study. Farmers who perceived animal stress as an important health issue in their herd reported problems of low feed quality, water shortage, bloat and hygiene in the shed and around the farm. This presumably indicated that these farmers were well aware of sanitation and hygiene of animals in their herds. That Clostridial vaccination is a protective factor for BLV infection is an interesting finding, given that vaccine administration is often done with common needles, and would therefore be expected to represent a potential risk for BLV infection. A plausible explanation

for this finding may be the in absolute terms low prevalence of BLV infection in infected herds. Also, subcutaneous administration of vaccine is likely to represent a reduced risk (Hopkins *et al.,* 1997). Lastly, the role of using concentrate as a feed in association with BLV infection cannot be explained on the basis of this data. It is possible that it was an indicator of the level of intensity of production and thereby the health awareness of the manager.

Both, stepwise logistic regression analysis and path analysis use the same statistical methods, but the latter will provide a better understanding of the causal relationships associated with the presence of BLV infection in dairy herds. In an outbreak of EBL in Spain, path analysis was used to explain the causal web of disease occurrence (Casal *et al.,* 1990). Casel *et al.,* (1990) found that herd size was highly correlated with the presence of infection, mainly through the indirect effect of the introduction of animals into the herd. As path analysis allows a lot of flexibility mainly during the process of constructing the null hypothesis model, this can result in many different models and interpretations.

Unlike logistic regression, which uses the same data set to quantify the contribution of risk factors at each step, classification tree procedures use different subsets of data to select a variable's best split between cases and controls. Therefore, a different set of predictors was identified in this analysis when compared with the regression-based methods. However, the advantage of this technique is that it provides a rule-based diagram based on the selected predictor variables. It has also better mechanisms for dealing with missing values than any of the regression techniques. The rules, as long as they are based on easily measurable information, can be incorporated into a decision support system for classification of BLV infection risk. It is also possible to directly incorporate differential weighting of risks of false positive and false negative classifications during the process of tree development. Finally, we used multiple correspondence analysis to define a multivariable descriptive graphical profile of herd risk factors associated with the presence of BLV infection. It was found that farmers from infected farms were relatively young (25-39 years), graduated from formal agricultural training programmes, had a recently established herd (1-3 years), had larger herds (>200 cows) and had bought-in animals. Thus, these variables could be used to describe the typical farmer managing a BLV-positive herd as a 'progressive farmer'. Herd

managers matching this profile should be targeted for education and extension activities regarding the control of BLV in dairy herds.

When analysing data with a large number of independent variables, the application of several multivariable techniques may be necessary to obtain a comprehensive impressions of potential cause-effect relationships. Investigators also need to integrate the results of their statistical analyses with their existing knowledge in order to explain any cause-effect relationships. The findings from this study have provided a system for classifying high risk and low risk farms, and it has been possible to identify specific management practices, which increase the risk of BLV infection at the herd level. As a next step, within BLV-positive herds it will be necessary to identify differences between low and high prevalence herds. The latter could be acting as the main source of infection for other herds. The herds with low prevalence are the ones which are more difficult to detect by the testing scheme applied as part of the national eradication programme. Hence, knowing their characteristics may become helpful during the final stages of eradication.

Conclusion

In this case-control study, the two predictors for BLV infection that consistently appeared in all analyses were herd size and the purchase of stock from external sources. Both risk factors can be used to explain transmission between farms. The number of years since farm establishment was also an important factor further emphasising the risk of infection resulting from animal purchases. In addition, performing pregnancy testing at the end of the season, having pedigree Friesian stock, not using preventive measures to prevent disease spread when mechanically dehorning were risk factors whereas having management stress was the only protective risk factor when looking at factors indicative of BLV transmission within the herd. These findings indicated that management practices were strongly related to the risk of BLV infection. However, some of the within-herd risk factors are poorly understood. We suggest identifying risk factors with regard to the variation of prevalence and incidence in the infected farms in order to clarify their roles with respect to the presence of BLV infection. These findings from this study should be taken into account when refining the strategy of the National EBL Control Scheme.

Table 4-1: Codes and descriptions of significant variables obtained from the univariate analysis.

Abbreviation	Description	Abbreviation	Description
ALLPARAS	Used parasiticides	METAMIN	Metabolic and mineral deficiency problems
BLACKLEG	Clostridial vaccines used	MNGSTRESS	Other non-infectious problems (related to management stress)
BREDFRIE	Breed pedigree Friesian	NODISPREV	No method to prevent disease spread when using mechanical dehorning
BREDJER	Breed pedigree Jersey	OLDER4W	dehorn when calves older than 4 weeks
BULBEEF	Bulls obtained from beef breeders	ONFARM	Have qualification as on-farm training
BULDAIRY	Bulls obtained from dairy breeders	OTINFEC	Other infectious problems
BULFRI	Bulls obtained Friesian breed	OUTSIDE	Rear calves outside with shelter
BULJER	Bulls obtained Jersey breed	PADDOCK	Cows calve in paddock
BULLNPED	Bulls obtained non-pedigree	PDOMCROS	Have crossbreed as the predominant breed in herd
BUYLOCAL	Stock bought from local area	PDOMFRIE	Have Friesian as the predominant breed in herd
CALVAGE	Milking herd separated depending on time of calving and age	PDOMJER	Have Jersey as the predominant breed in herd
CALVOT	Send calves to other than slaughter	PENSEP	Rear calves in separate pens in shed
COLOSTR	Feed colostrum	PENUNSEP	Rear calves in shed with no separation into pens
CONCENTR	Feed concentrate	PREGEND	Pregnancy test at end season
CONTPREG	Use of contractors for pregnancy test	PREGOTHER	Pregnancy test at other time
COWGRAZ	Graze cows at grazing company	PREVDIS	Avoid disease when buying by preventive measures
COWLEASE	Graze cows at leased land	PRODUCTI	Separate milking herd by production management
COWRUN	Graze cows at own run off	REPLACE	Buy stock for replacement
DISHEALT	Separate milking herd by disease and health management	SIBMTOH	Bulls have been used in other herds
DISINFDEHO	Disinfect dehorner when using mechanical dehorning	SICGRMX	Cows mix with other farmers' stock
DRHEIFER	Send dairy heifers to other than slaughter	SICGROF	Mix cows with other farmers' stock
EARSEASON	Pregnancy test at early season	SICGRON	Graze other farmers' stock on the same property
EMPMILOT	Send empty milking cows to other than slaughter	SIGBCF	Number of farms buy cows from
FARMCONS	Have farm management people as a consultant	SIGBHF	Number of farms buy heifers from
FARMERPREG	Use farmers for pregnancy test	SIHGRMX	Heifers mix with other farmers' stock
FARMLOCAT	Number of years that herd has been at the current location	SMCRTM	Number of hours leave calves with cows after calving
FSICUPS	Number of sets of cups in the dairy herd	SMCVCIND	Induce cows to calve
FSITMAM	Number of hours to milk cows at peak in the morning	SMHWHO	Person who dehorn calves
FSITMPM	Number of hours to milk cows at peak in the evening	SMPTCPC	Percentage of adult herd is pregnancy tested
GIAGE	Age group of respondent	SMPTUST	Use ultrasound for pregnancy test
GIWHO	Person who response the questionnaire	SMPTVET	Number of time veterinarians visit farms
HAVPEDFRIE	Have pedigree Friesian	SPRINGGR	Cows calve in springing group

Abbreviation	Description	Abbreviation	Description
HERDSIZE	Maximum number of milking cows in 1996/97 season	TECHAGR	Have qualification as agricultural technique
HFLEASE	Graze heifers at leased land	TYPEMANAGE	Person responsible for the management of herd
HISTORYD	Avoid disease when buying by history & certificate & inspection	UNKNOWN	Obtain bulls from unknown sources
HOMOMAS	Use homeopathic control for mastitis treatment	VARSOUR	Obtain bulls from many sources
INCREASE	Buy stock for increase in herd size	VETCONS	Have veterinarian as a consultant
LESS4W	dehorn when calves younger than 4 weeks	VETPREG	Use veterinarians for pregnancy test
MASMILK	Feed mastitis milk	WTHMILK	Feed withheld milk
MECHDEH	Use of mechanical dehorning	YSIBR	Use of Infectious Bovine Rhinotrecheitis vaccine

Table 4-2: Significant variables associated with the presence of BLV infection in a logistic regression analysis

Explanatory variable	Regression coefficient (SE)	P	OR (95% CI)
BLACKLEG [a]			
Clostridial vaccine used	-0.7069 (0.3110)	0.023	0.5 (0.3 - 0.9)
BULLNPED [b]			
Obtain non-pedigree bulls	0.8186 (0.4229)	0.0529	2.3 (1.0 - 5.2)
Obtain pedigree bulls	-0.0645 (0.3583)	0.8571	0.9 (0.5 - 1.9)
Don't use bulls at all	-1.1281 (1.369)	0.4100	0.3 (0.0 - 4.7)
BUYLOCAL [c]			
Buy stock from local province	0.3255 (0.2458)	0.1854	1.4 (0.9 - 2.2)
Buy stock from other provinces	1.0566 (0.3650)	0.0038	2.9 (1.4 - 5.9)
CONCENTR [d]			
Feeding concentrate	-0.8659 (0.3089)	0.0051	0.4 (0.2 - 0.8)
FARMLOCAT [e]			
Farm established <=3 yr	0.9896 (0.3450)	0.0041	2.7 (1.4 - 5.3)
Farm established 4 - 18 yr	0.8855 (0.3090)	0.0042	2.4 (1.3 - 4.4)
Farm established 19 - 25 yr	0.1636 (0.4371)	0.7082	1.2 (0.5 - 2.8)
HAVPEDFRIE [f]			
Have pedigree Friesian	0.6871 (0.2530)	0.0066	2.0 (1.2 - 3.3)
Have pedigree other breeds	-0.0099 (0.3351)	0.9763	1.0 (0.5 - 1.9)
HERDSIZE [g]			
Herd size more than 200 cows	0.8287 (0.2225)	0.0002	2.3 (1.5 - 3.5)
MNGSTRESS [h]			
Have management stress diseases	-0.9309 (0.2662)	0.005	0.4 (0.2 - 0.7)
NODISPREV [i]			
No preventive measures when dehorning	0.9300 (0.3461)	0.0072	2.5 (1.3 - 5.0)
Use other method for dehorning	-0.0726 (0.2610)	0.7811	0.9 (0.6 - 1.6)
PREGEND [j]			
Pregnancy test at the end of season	1.1628 (0.3455)	0.0008	3.2 (1.6 - 6.3)
Pregnancy test at other time	0.4453 (0.3366)	0.1858	1.6 (0.8 - 3.0)

[a] reference category: don't use Clostridial vaccine
[b] reference category: bull not obtained from external sources
[c] reference category: don't purchase stock
[d] reference category: no concentrate feeds used
[e] reference category: herds established > 25 years
[f] reference category: pedigree stock not present on farm
[g] reference category: herd size less than 200 cows
[h] reference category: no predominant health disorders perceived to be present
[i] reference category: no hygiene methods applied at time of dehorning
[j] reference category: no pregnancy testing undertaken

Table 4-3: Significant variables associated with the presence of BLV infection in a path analysis.

Explanatory variable	Outcome	Regression coefficient (SE)	P	OR (95% CI)
FARMERAGE	FARMEDU			
Age 25 - 39 yr		0.5088 (0.9142)	0.58	1.7 (0.3 - 10)
Age 40 - 54 yr		1.1151 (0.9162)	0.22	3.1 (0.5 - 18)
Age >=55 yr		2.762 (1.045)	0.01	16 (2.0 - 100)
FARMERAGE	FARMLOCAT			
Age 25 - 39 yr		1.8677 (0.9451)	0.05	6.5 (1.0 - 42)
Age 40 - 54 yr		0.7203 (0.9419)	0.44	2.1 (0.3 - 13)
Age >=55 yr		-0.3497 (0.9717)	0.72	0.7 (0.1 - 4.7)
FARMEDU	TYPEMANAGER			
Technical college		0.5752 (0.2604)	0.03	1.8 (1.1 - 3.0)
Diploma		-0.2002 (0.3452)	0.56	0.8 (0.4 - 1.6)
Bachelor		0.8088 (0.4470)	0.07	2.3 (0.9 - 5.4)
Other		0.7806 (0.5710)	0.17	2.2 (0.7 - 6.7)
TYPEMANAGER	HAVPEDFRIE			
Manager	1. Have pedigree	-0.0564 (0.3547)	0.87	1.0 (0.5 - 1.9)
Sharemilker	Friesian vs. don't	0.5239 (0.2166)	0.02	1.7 (1.1 - 2.6)
Other	have pedigree	0.8190 (0.6207)	0.19	2.3 (0.7 - 7.7)
Manager		-0.4405 (0.5152)	0.39	0.6 (0.2 - 1.8)
Sharemilker	2. Have pedigree	0.2969 (0.2806)	0.29	1.4 (0.8 - 2.3)
Other	other breeds vs. don't have pedigree	-0.3270 (1.1090)	0.77	0.7 (0.1 - 6.3)
BUYSTOCK	EBL			
Buy stock from local and other areas		0.4759 (0.2311)	0.04	1.6 (1.0 - 2.5)
FARMLOCAT	BUYSTOCK			
Farm established <=3 yr	(Buy stock from local	1.7918 (0.2820)	0.00	6.0 (3.5 - 10)
Farm established 4 - 18 yr	and other areas)	0.7863 (0.2300)	0.00	2.2 (1.4 - 3.4)
Farm established 19 - 25 yr		0.1912 (0.3362)	0.57	1.2 (0.6 - 2.3)
FARMLOCAT	EBL			
Farm established <=3 yr		0.9175 (0.3268)	0.01	2.5 (1.3 - 4.7)
Farm established 4 - 18 yr		0.8029 (0.2944)	0.01	2.2 (1.3 - 4.0)
Farm established 19 - 25 yr		0.1121 (0.4332)	0.80	1.1 (0.5 - 2.6)
HAVPEDFRIE	EBL			
Pedigree Friesian		0.7891 (0.2636)	0.00	2.2 (1.3 - 3.7)
Pedigree other breeds		-0.3582 (0.3602)	0.32	0.7 (0.3 - 1.4)
HERDSIZE	PREGEND			
Herd size > 200 cows	1. Pregnancy test at end season vs. don't preg test	0.7787 (0.2645)	0.00	2.2 (1.3 - 3.7)
	2. Pregnancy test at other time vs. don't preg test	0.7306 (0.2469)	0.00	2.1 (1.3 - 3.4)
HERDSIZE	EBL			
Herd size > 200 cows		0.7640 (0.2199)	0.00	2.1 (1.4 - 3.3)
MNGSTRESS	EBL			
Have management stress diseases		-0.9339 (0.2600)	0.00	0.4 (0.2 - 0.7)
NODISPREV	EBL			
No preventive measures when dehorning		1.0286 (0.3400)	0.00	2.8 (1.4 - 5.4)
Use other method for dehorning		-0.0987 (0.2557)	0.70	0.9 (0.5 - 1.5)
PREGEND	EBL			
Pregnancy test at the end of season		1.3054 (0.3445)	0.00	3.7 (1.9 - 7.2)
Pregnancy test at other time		0.5026 (0.3332)	0.1315	1.7 (0.9 - 3.2)

Table 4-4: Category quantifications of the two-dimensional multiple correspondence analysis.

Variables	First dimension	Second dimension
Cases		
EBL+	-0.75	-0.08
Buy stock (BUY+)	-0.49	0.14
Herd size >200 cows (COW+)	-0.49	-0.25
Farm has established 1-3y, 4-18y	-1.05, -0.12	0.59, -0.56
Farmer's age 25-39y	-0.71	0.30
Farmer's education (tech., dip.,bachelor agriculture)	-0.82, -0.22, -0.68	0.35, -1.16, 0.09
Controls	0.42	0.04
EBL-		
Don't buy stock (BUY-)	0.58	-0.17
Herd size <200 cows (COW-)	0.52	0.26
Farm has established 19-25y, >25y	0.74, 0.88	-1.04, 0.74
Farmer's age <25y, 40-54y, >=55y	0.23, 0.44, 1.05	-1.30, -0.66, 1.77
Farmer's education (tech., dip.,bachelor agriculture)	0.31	0.10
Eigenvalues	0.3321	0.2155

Figure 4-1: Null hypothesis path model.

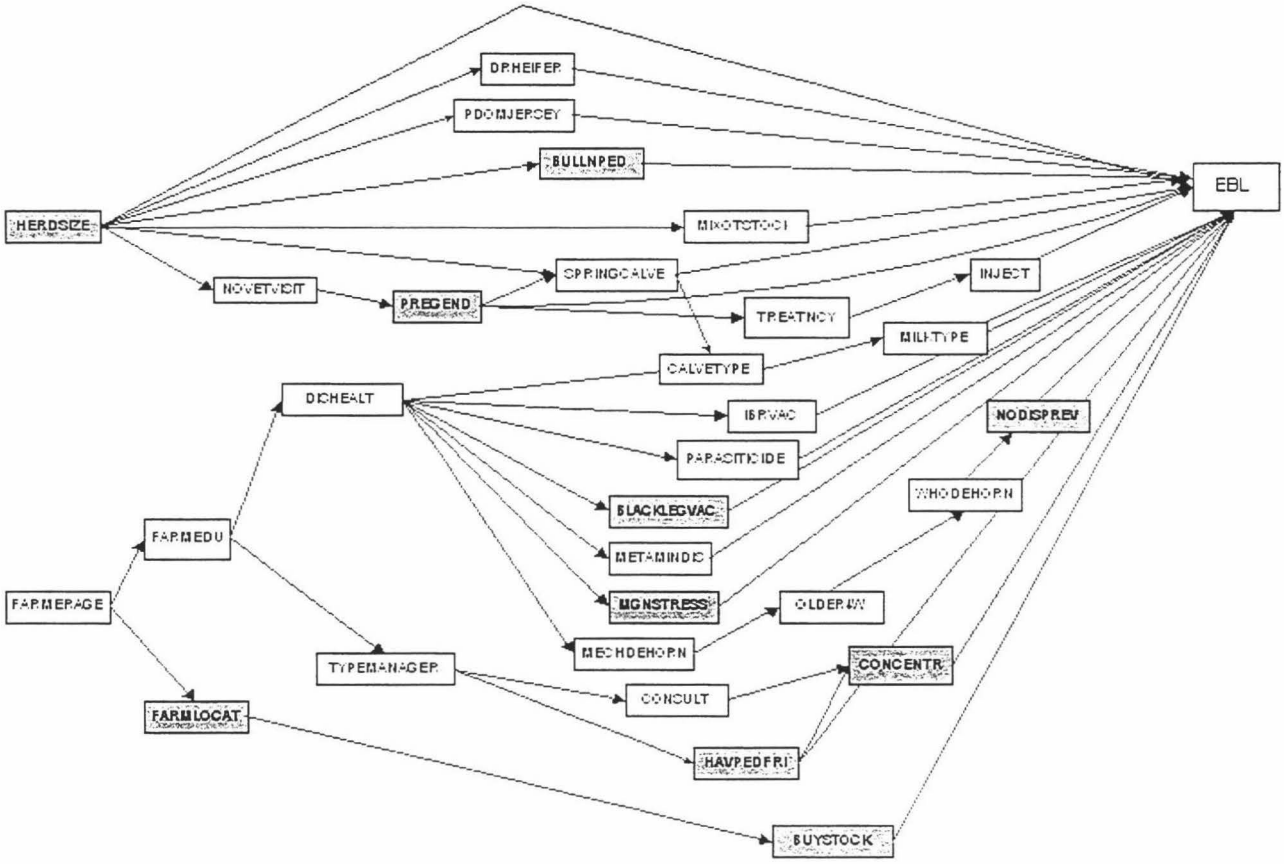


Figure 4-2: Final path model.

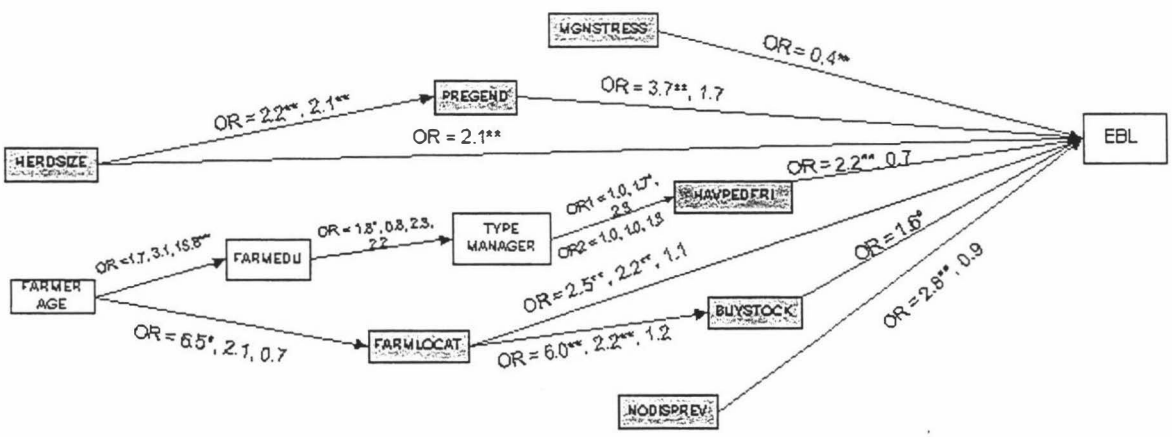


Figure 4-3: Classification tree for the presence of BLV infection in dairy herds. The numbers shown next to each node represent the number of cases and controls.

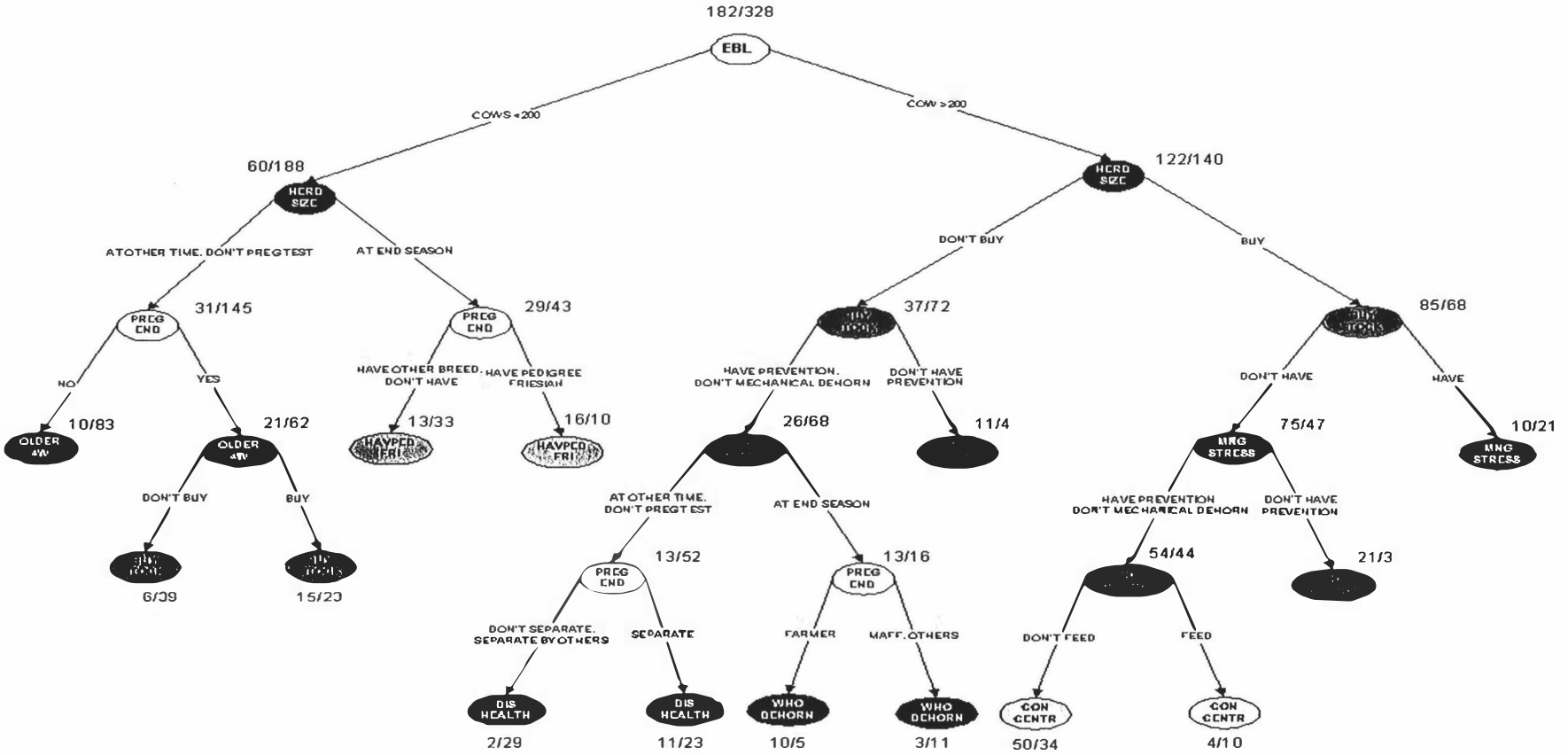
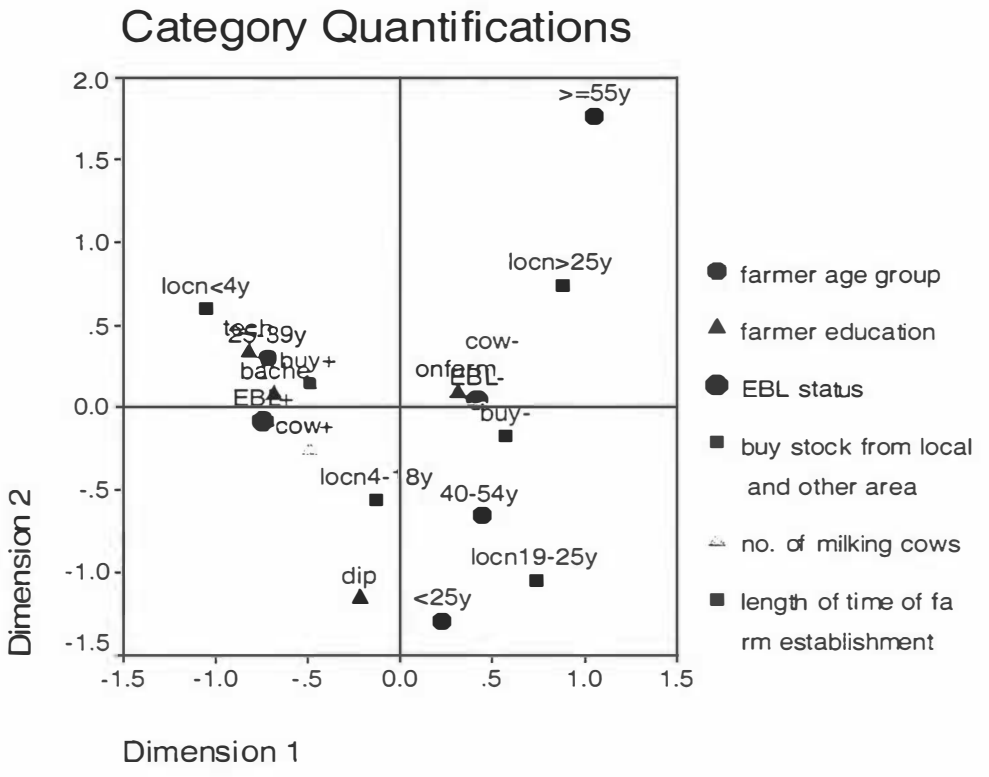


Figure 4-4: Category quantifications from the multiple correspondence analysis. Plus signs indicated yes or increase in the number of that factor. Minus signs indicate no or a decrease in the number of that factor. Percent axis inertia of dimension 1: 33%; dimension 2: 22%. Total inertia: 55%.



CHAPTER 5 -

A longitudinal study to identify risk factors for within-herd transmission of Enzootic Bovine Leukaemia Virus

Introduction

For the veterinary profession in New Zealand to provide accurate and cost-effective advice regarding BLV control, animal-associated risks and periods throughout the seasonal system of production that are associated with the highest risks of BLV transmission need to be identified. A knowledge of temporal and animal-associated risk factors allows BLV control programs to be better-focused thus reducing the overall costs of the program to the herd owner and minimising disruption of the husbandry routine for the herd manager.

While numerous studies identifying risk factors for BLV transmission have been conducted overseas (Bech-Nielsen *et al.,* 1978), (Onuma *et al.,* 1980) (Wilesmith *et al.,* 1980) (Thurmond *et al.,* 1982b), and (Lassauzet *et al.,* 1991b) there is variation among them regarding specific risks for BLV seroconversion. This is probably due to the different farming conditions in which the studies were conducted and factors such as climate, housing and local management practices. As a result of this, and due to the unique nature of the New Zealand dairy industry (where cattle calve seasonally and are grazed on pasture all-year round) it is not valid to extrapolate the results of overseas studies to New Zealand conditions. In New Zealand surveys of BLV status have periodically been made (Ministry of Agriculture & Fisheries 1979) (Hilbink 1991), (Hilbink *et al.,* 1993) and (Burton *et al.,* 1997) with between-herd prevalence ranging from 2.0 - 6.0% and within-herd prevalence ranging from 0.2 - 25% (Burton *et al.,* 1997). At the present time there are no published studies quantifying within-herd risks for BLV transmission in New Zealand.

The objectives of this study were to: 1) assess the precision of an ELISA test kit used to determine Bovine Leukaemia Virus (BLV) serostatus, (2) quantify the risk of vertical transmission, (3) identify temporal and animal-associated risk factors for BLV seroconversion in New Zealand dairy herds, and (4) determine whether there

were differences in the production capacity between seropositive and seronegative animals.

Materials and methods

On the basis of the 1996 BLV survey results (The New Zealand Dairy Industry 1997) four dairy herds in the Manawatu-Wairarapa region with a within-herd BLV prevalence of greater than 8.0% were approached to take part in a longitudinal population study of BLV incidence. The period of interest was 1 July 1997 to 30 August 1998.

Study population and data collection

Participant herd managers gave permission to access their herd's animal register data file maintained by the Livestock Improvement Corporation in its national database (Livestock improvement co-operation 1997). Data included on the national database for each herd included the biographical details of all female animals and, for animals that had calved at least once, selected event details associated with the current lactation. Biographical details included the unique animal identifier, the date of birth, breed, and details of the animal's sire and dam. Animal event details included dates and details of calvings and matings. This data was transferred to the dairy animal management program, DairyWIN version 98.1 (Massey University, Palmerston North, New Zealand).

For the duration of the study herd managers were asked to record the dates and details of husbandry practices related to all animals in the herd. These included treatments related to the induction of calving, reproductive examinations (including post-calving examinations and rectal examinations for pregnancy diagnosis) and management procedures such as parenteral medication, dehorning, vaccination and intradermal tuberculosis testing. Dates and details of calvings, breeding and removal from the herd were provided by quarterly updates to the animal register data file. Comparison of production among individuals was made using the 305-day lactation production estimates provided by the Livestock Improvement Corporation.

Throughout the study period herds were visited on four occasions when all female animals present in the herd (calves, heifers and millking cows) had a blood sample taken from the coccygeal vein. The timing of these visits was: (1) during the dry

period (July - August 1997), (2) after calving (November – December 1997), (3) after mating (March - April 1998), and (4) during the subsequent dry period (July - August 1998). One herd (herd 4) was unavailable for the first round of bleeding in July - August 1997 and entered the study at the second (November - December 1997) round of testing.

As antibodies to BLV infection develop for life, once adult animals were diagnosed as BLV-positive they were considered to be infected for the remainder of the study and hence not eligible for bleeding at the subsequent sampling. As a result of the possibility of passive immunity lasting up to twelve months after birth, all new-born calves were bled until the end of the study, regardless of BLV result.

At each visit a complete list of current animals in the herd was produced and at the time of bleeding each animal was verified against this list. As each animal was sampled details for that animal were quoted to the herd manager (lifetime identification, age, last calving date and reproductive status) to check the accuracy of the database records. Corrections were made if necessary.

After sampling the sera were separated from the samples and stored at -80° C. To identify BLV-infected cattle an Enzyme-Linked Immunosorbent (ELISA) diagnostic kit (Institut Pourquier, Montpellier, France) was used. Positive samples were determined on the basis of optical density (OD). If the optical density of a monowell ELISA test was in the range of 15% of a positive control it was regarded as suspicious and submitted for a second test using a biwell ELISA technique. Using the biwell technique, if the optical density was above 15% of the positive control, the sample was declared positive.

In the first round of sampling sera were tested individually. In the second and subsequent sampling rounds serum samples were randomly pooled into groups of ten. The pooled samples were tested using the monowell technique and if a positive result was obtained each serum sample was tested using the methodology described for the first round of testing. This methodology followed the approach adopted in the national control programme.

The ratio of seropositivity was obtained from standardization of the OD values with the OD value of positive control of each ELISA plate. Ratio of seropositivity was defined as the ratio of the actual OD recorded to the OD of the positive control.

Statistical analyses

The outcome of interest in this study was an animal changing from a negative BLV serostatus to a positive BLV serostatus. Three measures were used to describe the prevalence and incidence of BLV seroconversion in this study: point prevalence, cumulative incidence and incidence density. Point prevalence was used to characterise the number of cases that are known to have been present at a stated sampling date. Cumulative incidence (CI) was used to characterise the proportion of non-diseased individuals at the beginning of an intersampling interval that became seropositive during the period between samplings. Incidence density (ID) was used to characterise the rate at which new animals become seropositive between samplings, in relation to a specified number of animal-time units (e.g. cow-months).

The point prevalence of BLV serostatus at each sampling round was given by:

$$\text{Point prevalence} = \frac{n_{pos}}{(n_{total} - n_{cull}) + (n_{replace})}$$

Where:

n_{pos}: the number of BLV positive animals (known to be present at the herd sampling date),

n_{total}: the total number of cows sampled at the previous test,

n_{cull}: the number of cows removed from the herd between the two testing rounds, and

$n_{replace}$: the number of cows that entered the herd between the two testing rounds and were tested at the sampling.

A Fleiss quadratic 95% confidence interval in EpiInfo Version 6 (Epi Info Centre for Disease Control and Prevention, Atlanta, Georgia, USA) was used to determine the precision of the prevalence. Exact binomial 95% confidence intervals were calculated when the number of observations was less than five. Prevalence within age groups was calculated by taking the difference between the date of bleeding and an animal's birth date as the age of the animal.

The cumulative incidence of BLV seroconversion at each sampling round was given by:

$$\text{Cumulative incidence} = \frac{n_{pos}}{n_{neg}}$$

Where:

n_{pos}: the number of *new* BLV seropositive animals at the current sampling,

n_{neg}: the number of BLV seronegative animals in the previous round that were tested in the next round.

Incidence density was calculated as cases of BLV seroconversion per 100 cows per three month period at risk, with seroconversion assumed to have occurred at the midpoint of the three month interval. Incidence density was given by:

$$\text{Incidence density} = \frac{n_{pos}}{(n_{neg} + \frac{1}{2} n_{pos}) * i}$$

Where:

n_{pos}: the number of *new* BLV seropositive animals at the current sampling,

n_{neg}: the number of BLV seronegative animals at the previous sampling,

i: interval between samplings (in months).

For vertical transmission, the unit of interest was BLV status. A 2 *x* 2 contingency table was used to quantify the risk of BLV infection in calves born from infected and uninfected dams. The assumption was made that the serostatus of cows was determined when the cows were found positive to BLV infection while that of calves was determined by using the test result in the next sampling. This was because calf serostatus may be influenced by passive immunity particularly during the first six months after birth. Relative risk with 95% confidence intervals were calculated using Epi Info. Exact P values were calculated for differences between groups in dichotomous data and Fisher's exact test was used when cell sizes of less than five were present.

Generalized estimating equations using the GENMOD procedure in SAS for Windows (SAS Institute Inc., Cary, NC, USA) were used to quantify the risk factors for BLV seroconversion. BLV serostatus was the (binary) dependent variable and cow identifier, herd identifier and parity were included as independent variables. In

this analysis only cows of parity greater than and equal to 1 were used. Parity was re-coded into three groups: parity 1, parity 2 and parity 3 and greater. We assumed that the round of sampling had within-subject dependence and also checked for interaction terms.

For lactating females multivariate analysis of variance (MANOVA) in SPSS for Windows version 8.0 (SPSS Inc., Chicago, USA) was used to assess relationships between 305-day milk production and the fixed effect variables herd and BLV status. Parity was included as a covariate. In this analysis BLV status was defined as: (1) those animals that seroconverted at any time during the study period and (2) those animals that did not seroconvert during the study period. The null hypothesis was that BLV serostatus had no effect on production capacity. Potential two-way interaction terms were tested in the model. Pillai's trace was used to assess whether there were any differences with respect to the measurement of the different levels of an effect variables in any of the multiple dependent variables. The adjusted coefficient of determinant (R^2) was used to express the amount of variation in any of the production parameters explained by the effect variables.

Results

In total 2386 serum samples were tested and OD values ranged from –0.29 to >3.00. According to the recommendation of the testing procedure used we regarded a negative BLV result to be one in which the ratio of seropositivity ranged from 0 - 0.85 while a positive BLV result had a ratio of seropositivity of 1.15 or greater. A frequency distribution of the ratios of seropositivity displayed a binary peak which enabled clear differentiation between negative and positive results (Figure 5-1).

Descriptive statistics of the ratios of seropositivity for all samples are shown in Table 5-1. The mean ratio of seropositivity differed slightly between testing rounds (Figure 5-2) but were similar amongst herds (Figure 5-3). The second round of testing showed a slightly wider range of the ratio of seropositivity of positive results compared to other rounds.

Interpretation of BLV status in young calves is complicated by the presence of colostral antibodies to BLV. Counts of the number of samples that changed from BLV positive to BLV negative are shown in Table 5-2. Most calves were born between July and August and therefore had their first test in round 2. These calves

began to change serostatus in round 3 which was typically six months after birth. There were small numbers of calves that changed serostatus in round 4. The highest proportion of calves that changed serostatus was in herd 1 which had the highest overall prevalence of BLV.

Details of prevalence, cumulative incidence and incidence density stratified by testing round and herd are shown in Table 5-3 and Figure 5-4. At the first round of sampling the prevalence of BLV infection in herds 1, 2, 3 and 4 were 34%, 10%, 10% and 9%, respectively. Throughout the study CI ranged from 0 to 21 cases per 100 cows per 3 month period whereas ID ranged from 0 and 0.063 cases per 100 cow-months at risk. The highest incidence of BLV seroconversion was in herd 1 between rounds 1 and 2 (just prior to calving and immediately after calving) with a CI of 21 cases per 100 cows per 3 months and an ID of 0.063 cases per 100 cow-months at risk. The remaining three herds had CI range from 0 - 1.3 cases per 100 cows per 3 months and ID range from of 0 - 0.004 cases per 100 cows-months at risk.

Details of prevalence stratified by age group and herd are shown in Table 5-4 and Figure 5-5. Cumulative incidence stratified by age group and herd is shown in Table 5-5. In herd 1 BLV prevalence was highest (ranging from 34 to 46%). Peak prevalence was in animals of 1 to 2 years of age except in the first round of testing when peak prevalence was in animals 2 to 5 years of age. In herds 2, 3 and 4 (which all had similar BLV prevalence at the start of the study) the prevalence of BLV was variable until animals were two years of age. After this time prevalence plateaued.

Vertical transmission as a risk for infection is shown in Table 5-6. Calves born to BLV-positive dams were 3.7 to 44 times as likely to develop BLV infection as BLV-negative dams.

Results of the GEE analysis are shown in Table 5-7 and Table 5.8. Herd 4 and a parity of greater than 3 were chosen as the reference categories for the comparison of risk. Compared to herd 4, cows from herd 1 were at 4.4 times the risk of seroconverting to BLV throughout the study (Table 5.8). Cows of parity 1 and 2 were at 0.5 and 0.9 times the risk of seroconverting to BLV compared to cows of parity greater than 3 (Table 5-8).

The frequency of cows with positive BLV ELISA tests is shown in Figure 5-6. Seroconverted animals were animals which had positive results in the first sampling and remained seropositive until the study ended (these animals were coded as having a positive result for all sampling rounds) whereas seroconverting animals were animals that became seropositive from the second round onwards. The number of seroconverting animals was too small so we determined to assess production differences between infected and non-infected animals. Descriptive statistics for production stratifying by herd and BLV status are shown in Table 5-9 and Figure 5-7.

Multivariate tests of factors associated with production indices showed that herd, parity, BLV infection status and the interaction between herd and BLV infection status was associated with differences in 305-day milk production (see Table 5-10). Estimated marginal means for 305-day milk production (controlling for parity and herd) are shown in Table 5-11. BLV-positive cows in herd 1, 2 and 3 had higher production indices than those of BLV-negative cows.

Discussion

For the screening of large numbers of samples for BLV infection the ELISA test applied to pooled samples has been shown to be cost effective, easy to perform and precise (Mammerickx *et al.*, 1984) and (Hayes *et al.*, 1997). Care is required in interpreting the ELISA results of cows bled at or around the time of calving since changes in maternal immunological status (Johnson *et al.*, 1991a) result in higher numbers of results falling close to the suspicious range. Similarly, passive immunity to BLV can result in calves yielding false-positive results to BLV (Johnson *et al.*, 1991a). Where large numbers of stock are tested for BLV and eradication of the infection from herds is based on the removal of animals that return a positive ELISA test, there is the potential for unnecessary wastage of animals if care is not exercised in the interpretation of results, particularly if testing is carried out at or around the time of calving. The ELISA test used in this study provided clear differentiation of BLV-positive and BLV-negative samples in 99% of all samples tested. The range of OD results for positive samples varied between testing round with a slightly wider range of positive results found in the second round of testing (that is, when sampling was undertaken close to the calving period) (see Table 5-1 and Figure 5-2). We

conclude that the BLV ELISA test used in this study provided excellent precision at categorising BLV-positive and BLV-negative animals.

Romero (1983) in a study of 36 calves from a single research dairy unit found that the relative risk of calves acquiring BLV infection from milk was 0.59 (95% CI 0.16 - 2.19). In this study the relative risk of BLV from vertical transmission (that is both from *in utero* infection and infection via milk) was 16.0 (95% CI 7.3 - 35.0), although this risk varied markedly between the three herds considered (range 3.7 - 44.0). The contribution of milk consumption as a vehicle for BLV transmission is thought to be higher in this study compared with others as a result of the seasonality of calving where calves are fed with pooled milk, some of which may have been derived from BLV-positive animals. The relative risk of vertical transmission did not show a corresponding increase with herd BLV prevalence, indicating either differences in individual herd control practices for the feeding of BLV-infected pooled milk or insufficient study power to detect a difference.

In an eradication program there is a need to promptly diagnose BLV-positive animals and either remove them from the herd or institute programs to reduce the risk of spread of virus by these animals. Calves are a particular problem for this strategy because of the difficulty in differentiating between truly BLV-infected calves and those with transient BLV titres. In overseas studies BLV antibodies have been reported to persist for up to 12 months after birth (Ferrer *et al.,* 1981) (van der Maaten *et al.,* 1981a), and (Thurmond *et al.,* 1982c). In this study, where 187 calves were serially sampled every three months on three occasions, 18 calves that were found to be seropositive at the first testing had seroconverted by the second test (that is, at the third round of bleeding for the entire herd). One calf that was seropositive at the first test had seroconverted by the third test (that is, the fourth round of bleeding for the entire herd). The maximum age at seroconversion was 9 months of age. Thus, under New Zealand dairy farming conditions, we recommend that calves of greater than 9 months of age that return positive serology to BLV can be regarded as persistently infected.

Comparison of disease frequency in these herds revealed that incident BLV infections occurred most commonly within the first two years of life. Variation in the prevalence of each herd may be a result of differences in management practices which were performed on younger age groups such as mechanical dehorning,

drenching, and vaccination procedures. These practices can present the risk of exposure to BLV infection in a varying degree (Hopkins *et al.,* 1997). Our findings were in agreement with Thurmond (1983) who found that prevalence of BLV infection increased with age until the animals were 27 months old. To precisely observe risks for BLV infection is difficult, due to the requirement of frequent samplings and detailed recording of animal event information.

The magnitude of cumulative incidence and incidence density of positive BLV serostatus showed a direct relationship with the existing herd prevalence of infection. The herd with the highest prevalence of BLV (herd 1) had the highest CI and ID of infection. A logical explanation for these findings is that large numbers of BLV-positive animals in a herd (approximately 30% of which will show persistent lymphocytosis (Ferrer 1980) increase the opportunity for spread of infection. With respect to the very high incidence of seroconversion observed in parity 1 cows in herd 1 (see Table 5-5) we hypothesise that, in the absence of invasive management procedures applied to this group, the movement of this group of cows into a population of animals where the prevalence of BLV infection is high has resulted in high rates of disease transmission. Similar trends in incidence density in heifers has been identified in earlier prospective investigations (Thurmond *et al.,* 1983c).

In this study Generalised Estimating Equations were used to determine, for cows of parity 1 or greater, the relative importance of parity number and herd as risk factors for seroconverting to BLV. Herd carried the greatest risks for seroconversion and this may be explained by differences in physical farm facilities, management procedures and the method of conducting procedures which were unique to each herd manager.

Cows of parity 1 and 2 were at lower risk of seroconversion to BLV compared with cows of parity greater than 3 (see Table 5-8). A possible explanation for these findings is that, once cows have entered the milking herd, cumulative exposure to procedures such as pregnancy testing and the administration of parenteral medications increases the risk of BLV seroconversion with advancing age.

The result of the MANOVA showed that BLV infection had an effect on milk production and this effect was modified by the interaction between herd and BLV status. Since the number of herds used in this study was insufficient to differentiate

between those animals that were seropositive at the start of the study and those that seroconverted during the study, these results cannot be interpreted conclusively as to whether BLV infection can cause the difference in production indices. Furthermore, the extent of variation in the production parameters explained by the effect variables was small (9 to 10%). There may be important factors causing variation in these parameters which were not measured (Stevens, James 1996), which underlie the differences between BLV positive and negative cows. The effect of BLV infection on production capacity is a controversial issue. Langston *et al.* (1978); Huber *et al.* (1981); Jacob *et al.* (1991) and Heald *et al.* (1992) reported there were no significant trends in the production capacity between infected and non-infected cows. In addition, some of them found that there were no significant differences in the retention of these two groups as well. The most recent study in 102 herds revealed a negative association between herd-level milk production and BLV status but it was at a marginal level (OR 0.99) (Sargeant *et al.,* 1997b). An intervention study was also conducted by feeding a rich and poor diet to infected and non-infected cows. The results did not show significant differences but the sample size was too small (n = 16) (Brenner *et al.,* 1990). Thurmond *et al.* (1985); Brenner *et al.* (1989) and Pollari *et al.* (1993) demonstrated that seropositive cows were likely to be culled before seronegative cows. A study on two genetic lines selected for high and average milk production suggested that preferential culling might result from some reduced productivity or increased health problems with BLV infection and/or greater susceptibility to BLV infection was among average-producing cows (Detilleux *et al.,* 1991). Thus, a significant reduction in both milk yield and reproductive performance was the important reason resulting in the shorter lifespan. In contrast, Ming-Che *et al.* (1988) reported that the average lifespan of BLV-seropositive cows were longer than that of BLV-seronegative cows and production differences were due to high producers being older aged cows. This finding was in support of our results, indicating that the animals may be selected as high producers before they became infected and farmers tended to retain these animals in the herd. This culling strategy has the potential to hamper control efforts.

Conclusion

The presence of BLV infection can be diagnosed precisely by blood test using the ELISA technique. Vertical transmission may play an important role depending on the practice of calf rearing. New cases of BLV infection principally developed in animals within the first two years of age. Prevalence and cumulative incidence of disease was mainly influenced by the existing prevalence in the herd, rather than differences in management practices. The risk of seroconversion depended on factors at the herd level (i.e. related to management) rather than at the animal level. BLV-positive animals were more likely to have been higher producers compared to BLV-negative animals, but this may be due to the fact that these animals were retained in the herd because they were high producers.

Table 5-1: Mean and standard deviation of the ratio of seropositivity between positive samples and negative samples.

	Round 1		**Round 2**		**Round 3**		**Round 4**	
	BLV-positive	BLV-negative	BLV-positive	BLV-negative	BLV-positive	BLV-negative	BLV-positive	BLV-negative
$\bar{x} \pm$ S.D. (n)	1.8 ± 0.2 (112)	0.12 ± 0.1 (473)	1.7 ± 0.3 (123)	0.1 ± 0.1 (813)	1.6 ± 0.2 (63)	0.1 ± 0.1 (350)	1.7 ± 0.1 (28)	0.1 ± 0.1 (424)
	Herd 1		Herd 2		Herd 3		Herd 4	
	BLV-positive	BLV-negative	BLV-positive	BLV-negative	BLV-positive	BLV-negative	BLV-positive	BLV-negative
$\bar{x} \pm$ S.D. (n)	1.7 ± 0.2 (164)	0.1 ± 0.1 (331)	1.7 ± 0.3 (65)	0.1 ± 0.1 (589)	1.7 ± 0.3 (13)	0.1 ± 0.1 (218)	1.8 ± 0.3 (84)	0.1 ± 0.1 (922)

Table 5-2: Seroconversion from positive to negative serostatus in calves.

Herd	Round 2 (%)	Round 3 (%)	Round 4 (%)	Total (%)
1	0	10/58 (17.2)	1/39 (2.6)	11/97 (11.3)
2	0	1/29 (3.4)	0	1/29 (3.4)
3	0	0	0	0
4	0	7/61 (11.5)	0	7/61 (11.5)

Table 5-3: Prevalence, cumulative incidence and incidence density.

Herd	Disease frequency	Round 1	Round 2	Round 3	Round 4
1	Prevalence (%)	34 (73/214)	44 (116/265)	43 (107/246)	46 (108/236)
	CI per 3 months (%)		21 (30/143)	2.2 (3/135)	2.2 (3/136)
	CI per 9 months (%)				30.7 (35/114)
	ID		0.063	0.007	0.002
2	Prevalence (%)	10 (36/347)	10 (40/403)	7 (29/398)	7 (26/392)
	CI per 3 months (%)		0.3 (1/296)	1.2 (4/340)	0.9 (3/327)
	CI per 9 months (%)				2.3 (6/265)
	ID		0.001	0.004	0.003
3	Prevalence (%)	10 (10/105)	8 (12/155)	7 (11/149)	7 (11/149)
	CI per 3 months (%)		1.2 (1/85)	0.0 (0/138)	0.0 (0/138)
	CI per 9 months (%)				1.3 (1/80)
	ID		0.004	0.000	0.000
4	Prevalence (%)	-	9 (61/685)	9 (57/601)	10 (57/595)
	CI per 3 months (%)	-		1.3 (7/533)	0.6 (3/513)
	CI per 9 months (%)	-			1.5 (8/528)
	ID	-		0.004	0.002

CI: cumulative incidence
ID: incidence density

Table 5-4: Prevalence of BLV infection stratified by age group and herd.

Herd	Age group	Round 1		Round 2		Round 3		Round 4	
		Prevalence (%)	95% CI	Prevalence (%)	95% CI	Prevalence (%)	95% CI	Prevalence (%)	95% CI
1	0 - 6 m	0 (0/4)	0 – 60	35 (18/51)	23-50	N.A.	N.A.	N.A.	N.A.
	7 - 12 m	9 (5/53)	3 - 21	N.A.	N.A.	35 (18/51)	23-50	37 (18/49)	24-52
	>1 - 2 yr	23 (9/39)	12 - 40	62 (34/55)	48-75	68 (36/53)	54-80	65 (36/55)	51-77
	>2 - 5 yr	54 (40/74)	42 - 66	39 (39/100)	29-49	34 (29/86)	24-45	36 (29/80)	26-48
	>5 - 8 yr	41 (11/27)	23 - 61	46 (18/39)	30-63	45 (17/38)	29-62	49 (18/37)	32-65
	>8 yr	47 (8/17)	24 - 71	39 (7/18)	18-64	39 (7/18)	18-64	47 (7/15)	22-73
	Total	34 (74/214)	28 - 41	44 (116/265)	38 - 50	43 (107/246)	37 - 50	46 (108/236)	39 - 52
2	0 - 6 m	0 (0/9)	0 - 34	22 (5/23)	8 - 44	40 (6/15)	17 - 67	0 (0/18)	0 - 19
	7 - 12 m	20 (1/5)	1 -72	0 (0/17)	0-20	6 (1/17)	0-29	28 (7/25)	13-50
	>1 - 2 yr	5 (2/38)	1-18	3 (1/30)	0-17	3 (1/30)	0-17	3 (1/34)	0-15
	>2 - 5 yr	10 (11/114)	5-17	9 (14/151)	5-15	6 (8/141)	5-15	7 (9/124)	4-14
	>5 - 8 yr	12 (12/104)	6-20	13 (13/104)	7-21	7 (8/114)	3-14	4 (4/104)	1-10
	>8 yr	12 (9/75)	6-22	9 (7/78)	4-18	6 (5/81)	2-14	6 (5/87)	2-14
	Total	10 (35/347)	7-14	10 (40/403)	7-13	7 (29/398)	5-10	7 (26/392)	5-10
3	0 - 6 m	N.A.	N.A.	0 (0/19)	0-18	N.A.	N.A.	N.A.	N.A.
	7 - 12 m	N.A.	N.A.	0 (0/5)	0-52	0 (0/19)	0-18	0 (0/19)	0-18
	>1 - 2 yr	0 (0/1)	0-98	0 (0/30)	0-12	0 (0/27)	0-13	0 (0/22)	0-15
	>2 - 5 yr	10 (5/51)	4-22	13 (6/45)	6-27	13 (6/45)	6-27	12 (6/49)	5-25
	>5 - 8 yr	11 (3/27)	3-30	12 (3/25)	3-31	9 (3/34)	2-24	9 (3/32)	2-25
	>8 yr	8 (2/26)	1-25	11 (3/28)	2-28	8 (2/24)	1-27	7 (2/27)	1-24
	Total	10 (10/105)	5-17	8 (12/155)	4-13	7 (11/149)	4-13	7 (11/149)	4-13
4	0 - 6 m	-	-	7 (8/111)	3-14	N.A.	N.A.	N.A.	N.A.
	7 - 12 m	-	-	5 (5/100)	2-12	7 (8/114)	3-14	7 (8/115)	3-14
	>1 - 2 yr	-	-	5 (5/100)	2-12	6 (6/99)	2-13	6 (5/90)	2-13
	>2 - 5 yr	-	-	7 (19/283)	4-10	9 (20/230)	5-13	8 (18/222)	5-13
	>5 - 8 yr	-	-	16 (18/115)	10-24	14 (15/106)	8-23	15 (13/86)	9-25
	>8 yr	-	-	14 (11/76)	8-25	15 (8/52)	7-29	16 (13/82)	9-26
	Total	-	-	9 (61/685)	7-11	9 (57/601)	7-12	10 (57/595)	7-12

Table 5-5: Cumulative incidence per 3 months stratified by age group and herd.

Herd	Age group	Round 1 - Round 2		Round 2 - Round 3		Round 3 - Round 4	
		Prevalence (%)	95% CI	Prevalence (%)	95% CI	Prevalence (%)	95% CI
1	0 - 6 m	50 (2/4)	7-93	N.A.	N.A.	N.A.	N.A.
	7 - 12 m	N.A.	N.A.	0 (0/29)	0-12	0 (0/39)	0-9
	>1 - 2 yr	60 (28/47)	44-73	11 (2/19)	1-33	10 (2/19)	1-33
	>2 - 5 yr	0 (0/51)	0-7	2 (1/55)	0-10	0 (0/49)	0-7
	>5 - 8 yr	0 (0/14)	0-23	0 (0/20)	0-17	5 (1/20)	0-25
	>8 yr	0 (0/9)	0-34	0 (0/12)	0-26	0 (0/9)	0-34
	Total	21 (30/143)	15-29	2 (3/135)	0-6	2 (3/136)	0-6
2	0 - 6 m	N.A.	N.A.	22 (2/9)	3-60	N.A.	N.A.
	7 - 12 m	0 (0/11)	0-28	0 (0/9)	0-34	0 (0/12)	0-26
	>1 - 2 yr	0 (0/10)	0-31	0 (0/25)	0-14	0 (0/31)	0-11
	>2 - 5 yr	1 (1/122)	0-4	2 (2/124)	0-6	3 (3/109)	1-8
	>5 - 8 yr	0 (0/78)	0-2	0 (0/102)	0-4	0 (0/95)	0-4
	>8 yr	0 (0/65)	0-6	0 (0/71)	0-5	0 (0/80)	0-5
	Total	0 (1/296)	0-2	1 (4/340)	0-3	1 (3/327)	0-3
3	0 - 6 m	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	7 - 12 m	N.A.	N.A.	0 (0/19)	0-18	0 (0/19)	0-18
	>1 - 2 yr	N.A.	N.A.	0 (0/27)	0-13	0 (0/22)	0-15
	>2 - 5 yr	2 (1/41)	0-13	0 (0/39)	0-9	0 (0/43)	0-8
	>5 - 8 yr	0 (0/21)	0-16	0 (0/31)	0-11	0 (0/29)	0-12
	>8 yr	0 (0/23)	0-15	0 (0/22)	0-15	0 (0/25)	0-14
	Total	1 (1/85)	0-6	0 (0/138)	0-3	0 (0/138)	0-138
4	0 - 6 m	-	-	-	-	-	-
	7 - 12 m	-	-	N.A.	N.A.	N.A.	N.A.
	>1 - 2 yr	-	-	0 (0/98)	0-4	0 (0/103)	0-4
	>2 - 5 yr	-	-	1 (1/89)	0-6	1 (1/83)	0-7
	>5 - 8 yr	-	-	2 (5/214)	1-5	1 (1/192)	0-3
	>8 yr	-	-	1 (1/90)	0-6	1 (1/70)	0-8
	Total	-	-	1 (7/533)	1-3	1 (3/513)	0-2

Table 5-6: Vertical transmission showing number of calves, relative risk (RR) of calves born from infected dams.

Herd	Type of calving	Serostatus	Calf +	Calf -	RR
1	Spring calving	Dam +	10	5	3.7 (1.6 - 9.0)
		Dam -	5	23	
2	Non-seasonal calving	Dam +	3	2	35 (4.4 - 270)
		Dam -	1	57	
3	Spring and Autumn calving	Dam +	0	1	NA
		Dam -	0	22	
4	Spring calving	Dam +	7	7	44 (5.9 -330)
		Dam -	1	87	

+: BLV positive
-: BLV negative
NA: insufficient data for analysis

Table 5-7: Generalized Estimating Equations showing regression coefficients, standard error as well as the risk of seroconversion associated with herd and number of parity when assuming no dependence amongst the outcomes.

Comparison	Regression coefficient (SE)	P	OR (95% CI)
Herd [a]			
1	0.33 (0.19)	0.08	1.4 (0.9 - 2.3)
2	-0.77 (0.19)	< 0.01	0.5 (0.2 - 0.9)
3	0.09 (0.19)	0.65	1.1 (0.5 - 2.2)
Parity [b]			
1	-0.42 (0.19)	0.02	0.9 (0.5 - 0.9)
2	-0.16 (0.17)	0.37	1.2 (0.6 - 1.2)

[a] reference category: Herd 4
[b] reference category: parity ≥ 3
Pearson Chi-square = 2547.68 and Value/DF = 0.999

Table 5-8: Generalized Estimating Equations showing regression coefficients, standard error as well as the risk of seroconversion associated with herd and number of parity when taking into account the dependence amongst the outcomes.

Comparison	Regression coefficient (SE)	P	OR (95% CI)
Herd [a]			
1	1.49 (0.25)	0.00	4.4 (2.7 - 7.3)
2	-0.79 (0.34)	0.02	0.5 (0.2 - 0.9)
3	0.07 (0.38)	0.85	1.1 (0.5 - 2.3)
Parity [b]			
1	-0.71 (0.32)	0.03	0.5 (0.3 - 0.9)
2	-0.16 (0.27)	0.55	0.9 (0.5 - 1.4)

[a] reference category: herd 4
[b] reference category: parity ≥ 3

Table 5-9: Descriptive statistics of 305-day milk production stratified by herd and BLV status.

Herd	Milk (L)	Fat (kg)	Protein (kg)	Milksolids (kg)
Herd 1				
Total (n = 121)	2789 ± 963.3	117 ± 41.7	94 ± 32.3	211 ± 73.3
Infected animals (n = 51)	3262 ± 824.5	141 ± 35.3	110 ± 27.3	251 ± 61.5
Non-infected animals (n =70)	2444 ± 913.7	100 ± 37.7	81 ± 30.5	182 ± 67.7
Herd 2				
Total (n = 224)	3341 ± 1406.3	133 ± 57.2	105 ± 44.6	238 ± 100.3
Infected animals (n = 23)	3711 ± 1159.3	147 ± 44.2	116 ± 36.6	263 ± 80.1
Non-infected animals (n = 201)	3299 ± 1428.2	131 ± 58.3	104 ± 44.6	236 ± 102.1
Herd 3				
Total (n = 83)	2319 ± 1558.3	103 ± 70.4	81 ± 54.0	184 ± 123.7
Infected animals (n = 11)	2620 ± 1564.2	110 ± 64.6	90 ± 52.6	200 ± 116.8
Non-infected animals (n = 72)	2272 ±1563.2	101 ± 71.6	80 ± 54.5	181 ± 125.3
Herd 4				
Total (n = 453)	3268 ± 798.3	142 ± 36.7	109 ± 26.7	251 ± 62.5
Infected animals (n = 61)	3303 ± 822.2	143 ± 37.2	109 ± 28.3	252 ± 64.4
Non-infected animals (n = 392)	3262 ± 795.5	142 ± 36.8	109 ± 26.5	251 ± 62.3

Table 5-10: Multivariate tests showing predictors that had an effect on production capacity.

Effect	Value (Pillai's Trace)	F	Hypothesis df	Error df	P
Intercept	0.57	385.83[b]	3.0	870	0.00
Lactation number	0.01	2.65	3.0	870	0.05
Herd	0.11	11.07	9.0	2616	0.00
BLV status	0.01	3.73[b]	3.0	870	0.01
Herd x BLV status	0.02	1.9	9.0	2616	0.05

[a.] Computed using alpha = 0.05
[b.] R Squared = 0.102 (Adjusted R Squared = 0.093)
[c.] R Squared = 0.099 (Adjusted R Squared = 0.091)
[d.] R Squared = 0.082 (Adjusted R Squared = 0.074)
[e.] R squared = 0.093 (Adjusted R Squared = 0.085)

Table 5-11: MANOVA showing Estimated Marginal Means.

Dependent variable	BLV status	Mean (SE)	95% CI
Milksolids	Positive	242 (8.4)	225 - 258
	Negative	212 (3.8)	205 - 220

Figure 5-1: Frequency distribution of the ratio of seropositivity of all serum samples taken throughout the study.

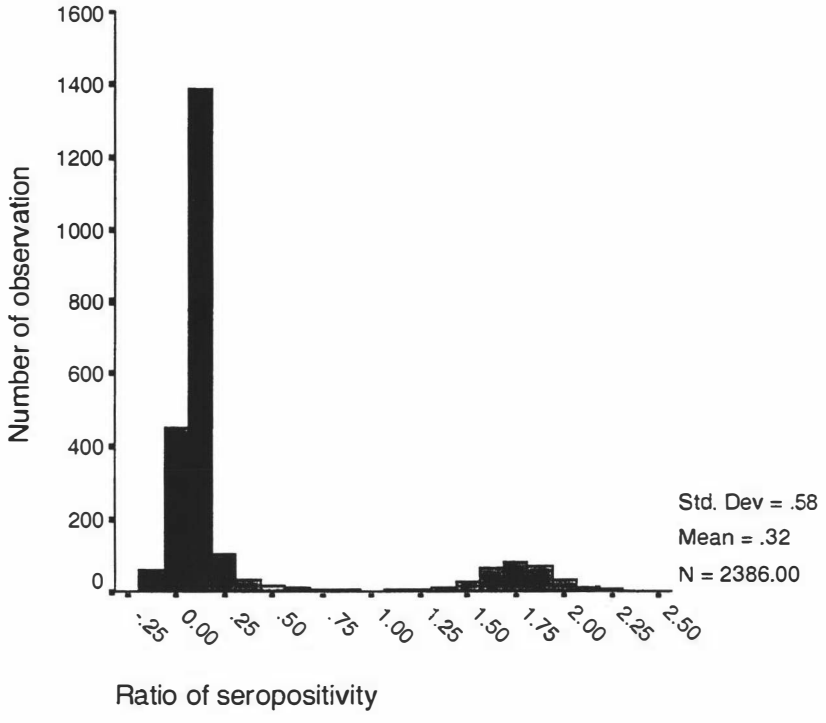


Figure 5-2: Frequency distribution of the ratio of seropositivity of all serum samples stratified by sampling round.

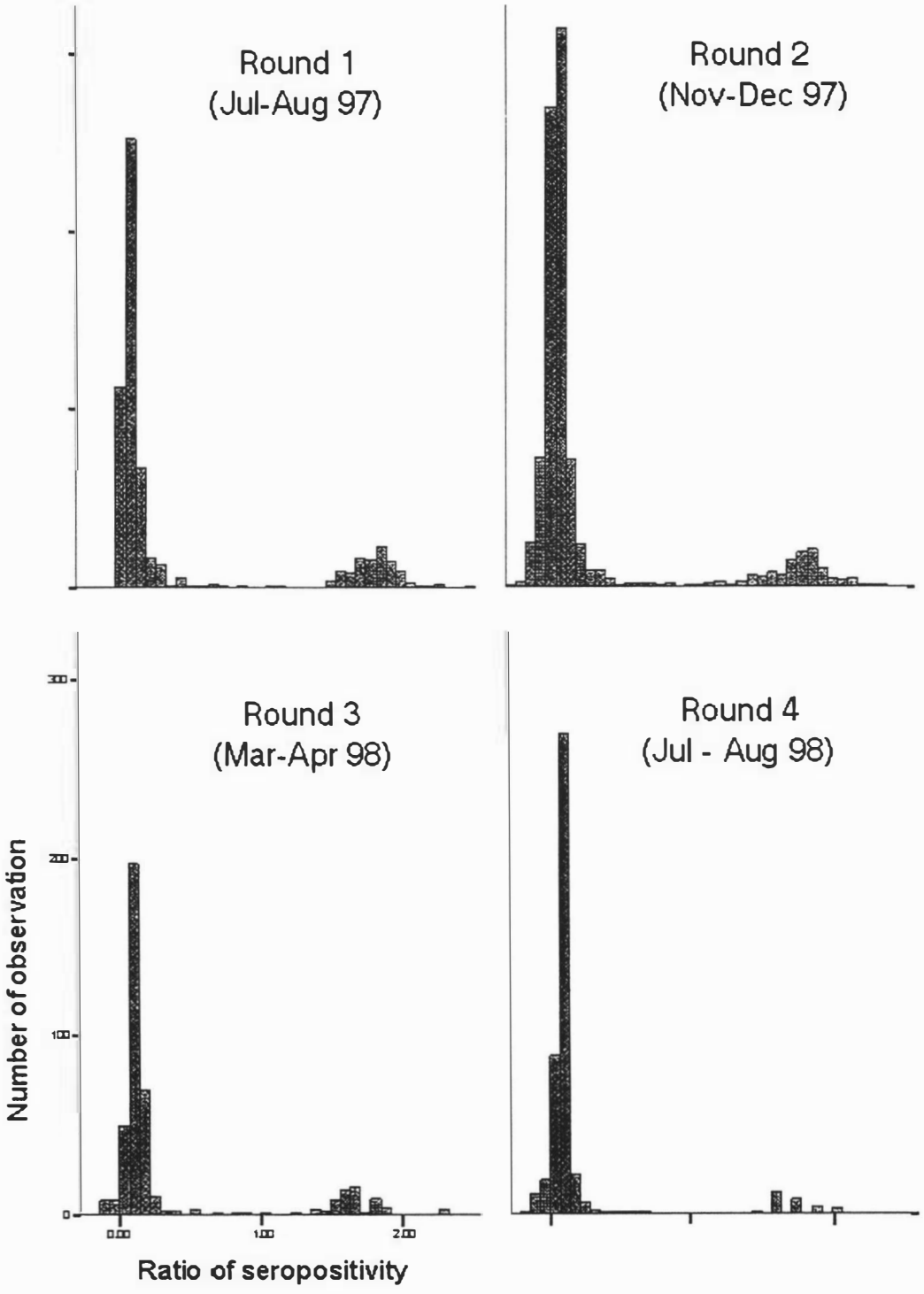


Figure 5-3: Frequency distribution of the ratio of seropositivity of all serum samples stratified by herd.

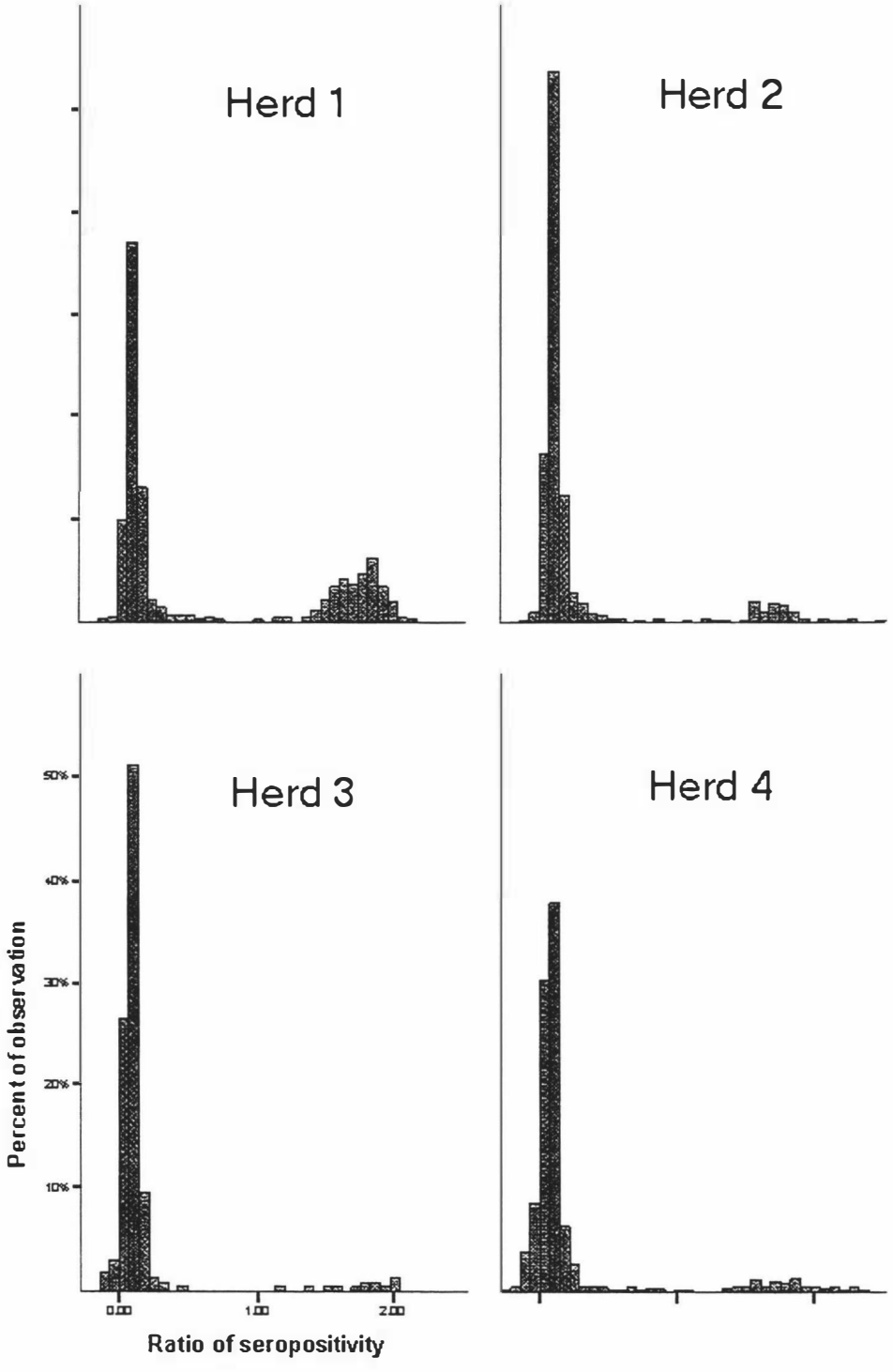


Figure 5-4: Prevalence of BLV infection in each herd at each sampling round.

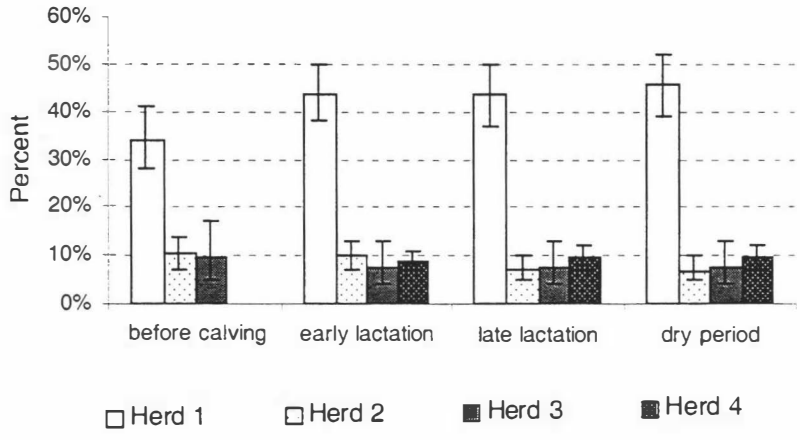


Figure 5-5: Prevalence of BLV infection in each herd stratified by age group in each round.

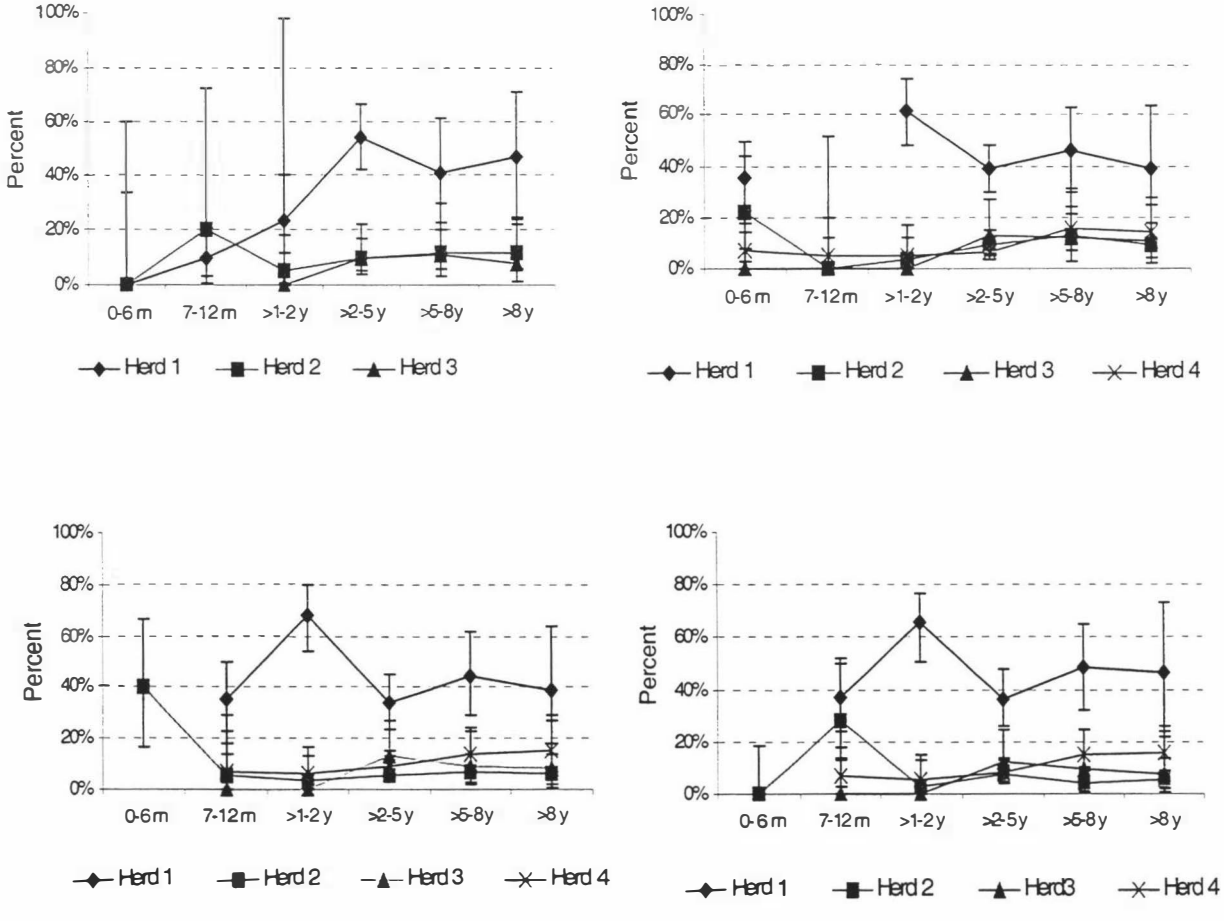


Figure 5-6: Frequency distribution of BLV serology results for all animals sampled.

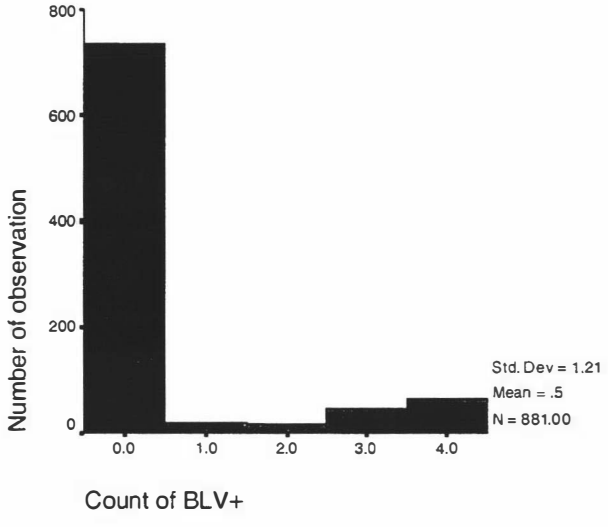
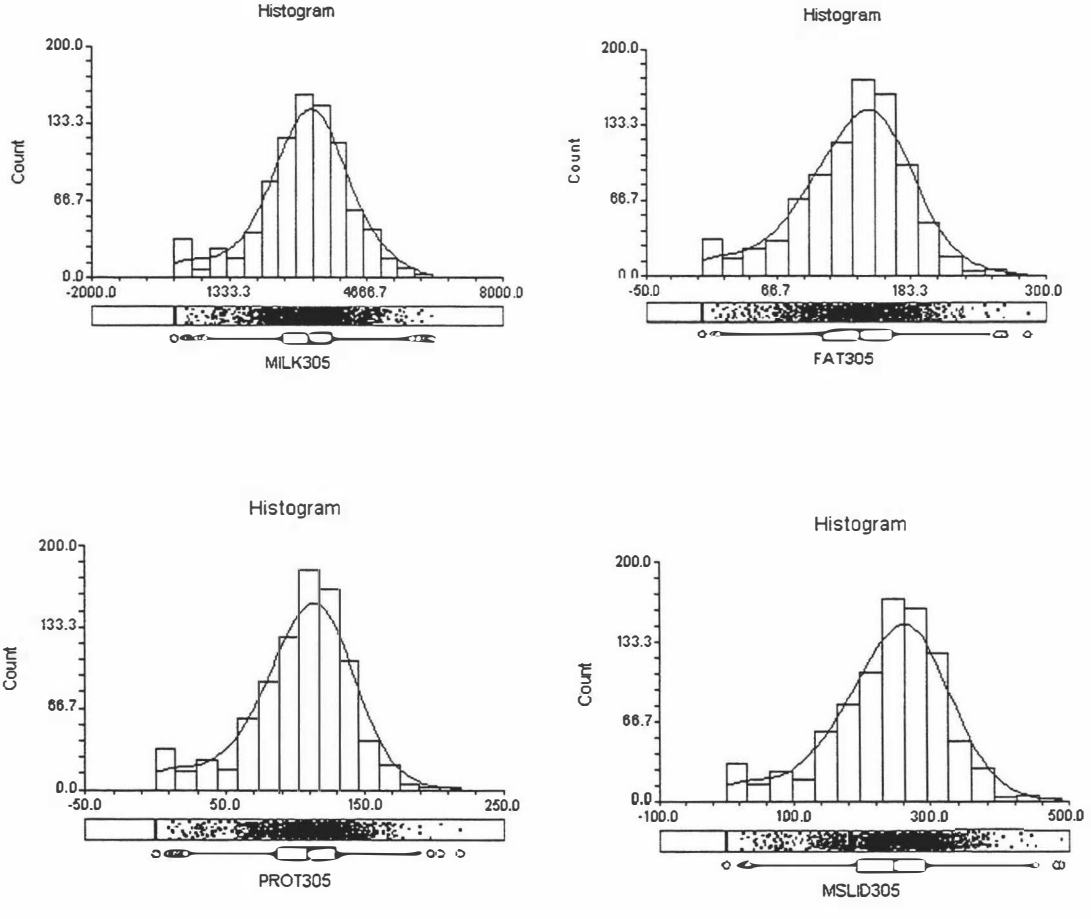


Figure 5-7: Histogram of 305 day milksolids yields for each of the four herds.



CHAPTER 6 - General Discussion and Conclusion

Epidemiology is the investigation of disease in populations and of factors that determine its occurrence, including assessment of other health-related events, notably productivity (Thrusfield, M 1995). Epidemiologists use various techniques including field studies, descriptive analysis, quantitative analysis and simulation modelling. Some of these techniques have been applied in this dissertation to understand the epidemiological pattern of Bovine Leukemia Virus infection in dairy cattle in New Zealand, and to identify causal and contributory factors involved in the disease.

Although the epidemiology of EBL and/or BLV infection has been extensively studied overseas (see Chapter 2) the findings of studies conducted overseas may not be directly applicable to New Zealand due to the unique system of dairy production in operation here. We investigated the presence of BLV infection by using two study designs. Firstly, the case-control study was used to identify risk factors and secondly, a longitudinal study to investigate the temporal pattern.

Study design

In the case-control study, an attempt was made by using questionnaire information from 719 farms (approximately 5% of dairy farms in New Zealand). The questionnaire contained 400 variables including farm specific information, demographic of herd, stock management, and information concerning the characteristics of the owner/manager. Misclassification between cases and controls was unlikely owing to the herd testing system used.

In the longitudinal study the total number of animals observed was approximately 1600 cattle from four herds. These four herds were quite different in many aspects, such as size, type of calving system and herd management style, according to the characteristic of the various farmers.

Results

In the case control study, we used a number of methods including univariate and multivariable techniques to identify and quantify the risk factors associated with the presence of BLV infection. Herds of large size, newly-established herds and the practice of purchasing stock off farm were strongly associated with the presence of BLV infection. In addition, path analyses assisted in explaining some of the underlying interrelationships between a number of risk factors and the presence of BLV infection.

In the longitudinal study, we collected blood samples four times at three-month intervals. Although animals were tested infrequently (every three months) we were able to monitor changes in antibody titres closely. The results showed that the interpretation of antibody titres must be made cautiously in those animals that have recently calved, as well as in new born calves. Calves may be regarded as being persistently infected with BLV if they show a positive antibody response after 9 months of age.

Milk-borne infection was likely to play an important role in vertical transmission. This indicated that the practice of feeding discarded milk to calves should be avoided in infected herds.

In all herds most new infections occurred in animals which were under two years of age, so the focus for control of BLV transmission should be in calves and yearlings. A BLV control program should therefore involve eliminating the risk factors such as mechanical dehorning and other actions which could pass blood cells from one animal to another.

For the risk of a cow undergoing seroconversion, herd was the most important factor, suggesting that there were several factors which differed amongst the four herds, but which could not be examined within the longitudinal study. Lactation number was another factor involved with seroconversion. Animals on their first lactation were less likely to seroconvert than those on other lactations. This indicated that some risk factors were involved in older cows and were not practised in heifers.

The temporal pattern of BLV prevalence was likely to be a consequence principally of the overall intensity of exposure, which is determined by the existing herd prevalence whereas the incidence was likely to be based on both the same factor

together with the culling policy. This finding suggested that the studies on high prevalence and low prevalence farms should be pursued differently to understand the somewhat different mechanisms operating in the two situations.

Though we found that herd and BLV infection status were the factors differentiating production capacity in infected and non-infected animals, the conclusion can not be reached that BLV infection had an effect on production. Firstly, we did not assess the effect of BLV infection in seroconverting animals and secondly, this significant difference was likely to be a result of differences between farms due to involuntary culling on grounds other rather than the serostatus.

At the present time, a small number of high prevalence herds in New Zealand have been identified. We suggest that an extended longitudinal study should be conducted in these herds and focused on more closely in terms of frequent sampling, different handling of samples (change from pooled sample to individual sample diagnosis). Another alternative, the case control study nested in the longitudinal study would be worthwhile to compare risk factors with different study designs in the same herds. In the next step, we would recommend simulation modelling to predict the outcome. Lastly, intervention study would be worthwhile in testify these findings and hypotheses.

In summary, for New Zealand dairy herds, a control program for BLV infection should be focused on milk-borne transmission. Emphasis on adopting the changes on management practices should be placed on avoiding purchasing stock from external sources and changing blood-transferring practices together with using disinfectant.

APPENDIX 1

«TITLE» «INITIAL» «NAME»
«ADDRESS1»
«ADDRESS2»
«ADDRESS3»

Dear «TITLE» «NAME»

The New Zealand Dairy Board has initiated a major programme to eradicate the disease EBL (Enzootic Bovine Leucosis) from the national dairy herd. This has resulted in the development of the New Zealand Dairy Industry EBL Control Scheme - designed to help farmers identify and control the prevalence and spread of the disease within their own herds.

EBL is a slowly spreading viral disease which affects the immune system of cattle. It can cause cancer of the lymphatic system in up to 5% of infected animals. EBL reduces the resistance of cattle to other diseases and undermines productivity and longevity. The disease is regarded as being of no danger to human health.

EBL is common in the cattle populations of most dairying countries. Many of New Zealands major trading partners have EBL eradication schemes already in place, with some having removed the disease from their herds completely. International recognition of New Zealand dairy herds being declared 'EBL-Free' is expected to confer long-term marketing advantages for product and animal exports.

As part of the process of establishing greater knowledge about EBL, we are conducting a survey of 600 dairy farmers throughout the country to identify common farm management practices which may be a contributing factor to the infection and spread of the disease within herds. **Your assistance in completing and returning the enclosed survey form** will provide valuable information for formulating disease control recommendations for the New Zealand dairy industry.

Milk and blood testing for the presence of EBL will begin in the new year for all herds in the Taranaki, Bay of Plenty and Wellington / Hawkes Bay regions. These will be followed in the 1997/98 season by herds in all other regions.

Your help with this important industry project is greatly appreciated. For further information, please contact me at the address shown above.

Yours sincerely

PROJECT MANAGER

Survey Number: ***«SurveyNumber»***

EBL Survey

Please tick or circle the appropriate boxes. Y=Yes, N=No, ?=Don't know

I Farm Specific Information

1 What is the maximum number of cows milked in the 1996/97 season? ☐☐☐☐

2 Is this herd? ☐ spring calving
☐ autumn calving
☐ spring and autumn calving
☐ non-seasonal town supply herd

3 How many years has this herd been at the current location? ☐☐

4 What type of dairy shed do you have? ☐ rotary
☐ herringbone
☐ other, *please specify* _____

5 How many sets of cups do you have in your dairy shed? ☐☐

6 How many hours does it take to milk your cows at peak? am ☐☐ pm ☐☐
(From the first cow into the shed to the last cow out of the shed).

7 What person is responsible for the management of the herd day to day? Tick one
☐ owner
☐ manager
☐ 50% sharemilker
☐ 39% sharemilker
☐ 29% sharemilker
☐ other percentage sharemilker, *please specify* ☐☐ %
☐ salaried workers
☐ other

8 What do you think are the four most important animal health problems in your milking herd? Office use
1. _____
2. _____
3. _____
4. _____

9 What is the herds' average somatic cell count during peak production this season? ☐☐☐☐ ,000

II Stock Information

We would like some general information on your stock, especially purchases, sales and grazing off.

A General

1 Do you buy adult dairy cows? **Y** **N**
If you answered YES (Y) to question 1 then answer questions 1a and 1b.

1a From how many different farms have you purchased adult cows during the last two years? ☐☐

1b What breed of cows have you bought over the last two years?

(Tick as many as required)

- Pedigree Friesian ☐
- Pedigree Jersey ☐
- Pedigree Ayrshire ☐
- Friesian ☐
- Jersey ☐
- Ayrshire ☐
- Crossbreed ☐
- other *please specify* _____ ☐

2 Do you buy young dairy stock (less than two years of age)? Y ☐ N ☐

*If you answered NO (N) to **both** questions 1 and 2, please go to question 6.*

2a From how many different farms have you purchased young stock during the last two years? ☐☐

2b What breed of young stock have you bought over the last two years?

(tick as many as required)

- Pedigree Friesian ☐
- Pedigree Jersey ☐
- Pedigree Ayrshire ☐
- Friesian ☐
- Jersey ☐
- Ayrshire ☐
- Crossbreed ☐
- other *please specify* _____ ☐

3 What is the major reason for buying stock?

- increase in herd size ☐
- replacements ☐
- breeding ☐
- trading ☐

4 Where have you bought cattle from?

- local ☐
- other provinces in New Zealand ☐

5 With respect to the animals bought in, do you take measures to avoid the introduction of disease into your herd? Y ☐ N ☐

If you answered YES (Y) to question 5 please answer question 5a.

5a What are the measures you take?

Office use

_____

_____

_____

6 Do you separate your milking herd into different herd groups (apart from dry cows)? Y ☐ N ☐

If you answered YES (Y) to question 6 please answer question 6a.

6a What are the reasons for having different herd groups?

(tick as many as required)

- milk production ☐
- body condition ☐
- age ☐
- lameness ☐
- other disease ☐
- day of calving ☐
- cyclers / non-cyclers ☐

other

7 Did you send stock directly for slaughter last season? Y N

If you answered YES (Y) to question 7 please answer question 7a.

Please indicate no.

7a Were they? *(tick as many as applies)*

- milking cows (dry in-calf or milking)
- empty milking cows
- steers
- bulls
- dairy heifers (empty)
- beef heifers

8 Did you sell cows and stock for **other** than slaughter last season? Y N

If you answered YES (Y) to question 8 please answer question 8a.

Please indicate no.

8a Were they? *(tick as many as applies)*

- milking cows (dry in-calf or milking)
- empty milking cows for carryover
- steers
- bulls
- dairy heifers
- beef heifers
- calves
- weaners

9 Do you have any pedigree cows in your herd? Y N

If you answered NO (N) to question 9 please go to question 11.

9a Are they?

- Friesian
- Jersey
- Ayrshire
- Other *please specify*_____

10 Do you breed pedigree cows? Y N

If you answered YES (Y) to question 10 please answer questions 10a and 10b

10a Are they?

- Friesian
- Jersey
- Ayrshire
- Other *please specify*_____

10b Are these pedigree animals bred for sale as pedigree stock? Y N

11 Are there any dairy cows (culls carryovers etc) that are on the same property as the dairy herd but are not currently on the Animal Register? Y N

If you answered YES (Y) to question 11 please answer question 11a.

Please indicate no.

11a Are they?

- cows to cull
- carryover cows
- other

B Cows

1 Have you grazed or leased any cattle, belonging to other farmers, on your dairy farm in the last two years? Y N ?

2 Have you grazed cows off the farm during the last two years? Y N

If you answered NO (N) to question 2 please go to Section C, Heifers.

2a Where have these animals been grazed off?
- own run off
- leased land
- grazing company
- other *please specify* _____

3. Have the animals been mixed with any other farmers stock when grazed off? Y N ?

C *Heifers*

1 Do you graze heifers off the farm? Y N

If you answered NO (N) to question 1 please go to Section D.

1a Where are these animals grazed off?
- own dry block
- leased land
- grazing company
- other contract grazing

2 Are the heifers mixed with other farmers stock (apart from bulls) when grazed off? Y N ?

D *We would like to know some details about the service bulls used over your milking cows.*

1 How many weeks do you use AB for? ____

2 Do you use bulls at all? Y N

(if No (N), go to Section III, Stock Management)

2a How many weeks do the bulls usually stay in the herd after AB finishes? ____

3 Do you obtain bulls from off the farm? Y N

(if No (N), go to question D6)

3a Are they?
- from the same supplier generally
- various sources

3b Where do they come from? *(please tick the major source)*
- dairy breeding herd
- beef breeder
- from the cheapest source
- any available animals
- saleyard

4 Have you bought dairy breed bulls in the last two years? Y N

If you answered YES (Y) to question 4 please answer question 4a.

4a Are they? *(tick as many as applies)*
- Pedigree Friesian
- Pedigree Jersey
- Pedigree Ayrshire
- Friesian
- Jersey
- Ayrshire
- other Dairy *please specify*_____

5 Do you know if the bulls have been used for mating in other herds? Y N ?

6 Are the bulls tested for fertility before use? Y N

If you answered YES (Y) to question 6 please answer question 6a.

6a Who tests them?
- the vet
- visually inspected by yourself
- other

III Stock Management

A Calf Rearing

1 How long do you leave calves with the cow after calving?
- 12 hours or less
- 12 to 24 hours
- 24 or more hours

2 Do you use nurse cows for feeding some or all calves ? Y N

3 What type of calf rearing facility do you have?
- shed with no separation into pens
- separate pens in shed.
  (What is the maximum number of calves per pen?) *No. of calves/pen* ☐☐☐
- outside with shelter
- individual rearing crates for each calf
- other *please specify*_____

4 What is the age of the calves at weaning (off milk)? *(Number of weeks)* ☐☐

5 Do you feed discard milk types to heifer calves? Y N

If you answered YES (Y) to question 5 please answer question 5a.

5a Which of the following types do you feed?
(tick as many as applies)
- colostrum
- mastitis
- milk from withheld cows (penicillin milk)
- milk containing blood
- other *please specify* _____

6 Are calves mixed with older stock? Y N ?

B Calving

1 Are cows calved?
- in paddock with rest of dry herd
- springing group
- on a feed pad or other grazing off area

C Pregnancy Testing

1 Do you pregnancy test your adult herd? Y N

If you answered NO (N), go to question C7

1a What percentage of the herd is tested?
- > 90 percent of herd
- > 15 and < 90 % (part)
- < 15 %

2 Do you use any ultrasound testing? Y N

3 Who do you use to pregnancy test the cows?
- veterinarian
- contractor
- do it yourself

4 When is pregnancy testing done? *(Please tick the correct time period))*
- as late as possible to be sure they are pregnant
- early as practical so all the herd can be tested at once
- early and late in the season
- dependant on when the empties need to be culled
- other *please specify*_____

5 How many times would the veterinarian visit your farm for reproductive work, including pregnancy testing, checking non-cyclers during a season? (Do not include heifer visits) ☐☐

6 Do you treat your non-cycling cows? Y N

If you answered YES (Y) to question 6 please answer question 6a

6a Are they injected with any drugs, for example, prostaglandins or oestrogens? Y N

7 Are yearling heifers pregnancy tested? Y N

If you answered YES (Y) to question 7 please answer question 7a.

7a What percentage of the heifers are tested?
- > 90 percent of herd ☐
- > 15 and < 90 % (part) ☐
- < 15 % ☐

D Vaccinations

1 What vaccinations are used in your adult herd? *(tick as many as applies)*
- leptospirosis ☐
- blackleg or "five in one" ☐
- johnes disease ☐
- hyper immune Stolle ☐
- Mucobovin (BVD) ☐
- other *please specify*_____ ☐
- none ☐

2 What vaccinations are used in young stock? *(tick as many as applies)*
- leptospirosis ☐
- blackleg ☐
- johnes disease ☐
- Mucobovin (BVD) ☐
- other *please specify*_____ ☐
- none ☐

3 What other drugs do you use for groups of 10 or more dairy stock (ie exclude individual sick animals)?

(tick as many as applies)
- vitamin injections ☐
- copper ☐
- selenium ☐
- antibiotics ☐
- injectable worm drenches ☐
- other *please specify*_____ ☐

4 How often do you change needles and syringes when treating a group of dairy stock on the same day?
- change for every animal ☐
- change sometimes ☐
- not at all ☐
- don't know ☐

5 Do you induce cows to calve? Y N

E Drenching

1 How many months of the year do you use daily drenching on your cows?

- none
- <1 month
- 1 to 3 months
- > 3 months

2 What methods of mastitis control do you use? *(tick as many as applies)*

- teat spray
- dry cow therapy
- culling
- treatment of clinical cases
- other *please specify*_____
- none

3 What supplementary feeds do you use? *(tick as many as applies)*

- silage
- hay
- turnips
- other forage crops
- concentrates
- other

F Dehorning

1 When do you dehorn animals?

- as calves less than 4 weeks
- as calves older than 4 weeks
- yearlings
- older
- not performed

2 What dehorning methods have you most often used in last 2 years? *(tick only one)*

- mechanical (scoop or cutters)
- electric cautery (disbudding)
- dehorning paste
- other

*If you answered **mechanical** for question 2 please answer questions 2a, 2b, and 2c*

2a What precautions are taken to prevent spread of disease?

- wash dehorners
- disinfect dehorners
- scrub
- none

2b Are the animals kept in the yards after dehorning? **Y** **N**

If you answered YES (Y)to question 2b then answer question 2c

2c How long are the animals kept in the yards?

- < 30 minutes
- 30 to 60 minutes
- longer

3 Who does the dehorning? *(tick only one)*

- own staff/self
- MAF
- veterinarian
- other

IV General Information on herd owner /manager

We would like to ask some questions about your background and working situation

1 Are you? The
- owner ☐
- sharemilker ☐
- employee ☐
- other ☐

2 What is the predominant breed in your herd?
- Friesian ☐
- Jersey ☐
- Ayrshire ☐
- Crossbreed ☐
- other ☐

3 Are you a member of a Breed Society? **Y** ☐ **N** ☐

4 In which of the following age groups do you belong?
- less than 25 ☐
- 25 to 39 ☐
- 40 to 54 ☐
- 55 or older ☐

5 What type of relevant qualifications do you have?
- on-farm training ☐
- technical agricultural institute ☐
- agricultural diploma ☐
- bachelor of agriculture ☐
- other ☐

6 Do you use the resources of a commercial animal or farm management consultant (paid adviser)? **Y** ☐ **N** ☐

If you answered YES (Y) to question 6 then answer question 6a

6a Who do you use?
- veterinarian ☐
- nutritionist ☐
- farm management consultant ☐
- other agricultural consultant *please specify*_____ ☐

THANK YOU FOR YOUR PARTICIPATION

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