Ruminant density, Verocytotoxigenic *Escherichia Coli* and cryptosporidiosis in New Zealand: Descriptive and ecological analyses

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A Dissertation presented in partial fulfilment for the degree of
Master of Public Health (MPH) at the
University of Otago, Dunedin

October 2010

Table of Contents

List of Figures

List of Tables

Acknowledgements

Michael Baker and Nigel French were the experts who helped me source data and assisted me with the study design. Katrina Sharples and Nigel Dickson were my excellent and encouraging supervisors who provided invaluable statistical, epidemiological and practical advice. Ruth Pirie of Environmental Science and Research helped me uplift the human health data. Robert Sanson, manager of the Agribase™ database, mapped appropriate data onto the 2006 meshblock pattern, and helped me to source and interpret the animal data. Staff at the Department of Preventive and Social Medicine in Dunedin provided encouragement and useful advice. In particular, Hilda Firth and John Holmes provided guidance in the early stages of the study. The Department also provided the funds necessary to access the human and animal data. The New Zealand College of Public Health Medicine provided an income stream. Fellows of the College including Douglas Lush provided valuable insights regarding the relevance of my research to policy. My fantastic wife, Amy, was understanding, firm and helpful at just the right moments. Her parents Michael and Stephanie Revell, together with my father Dennis Thorburn, assisted with proof reading. Finally, our daughters, Lucy and Audrey provided a delightful distraction.

Abstract

Background

Verocytotoxigenic *Escherichia coli* (VTEC) and cryptosporidiosis are emerging zoonotic infections. Intensive agricultural land use has been implicated in their emergence and sustained incidence. New Zealand has large numbers of dairy cattle, beef cattle, and sheep that may be reservoirs for VTEC and *Cryptosporidium*. The aim of this study was to assess the relationship between the density of the major farm animals in New Zealand (dairy cattle, beef cattle and sheep), and the rates of notified VTEC and cryptosporidiosis. To supplement this, the study also described the epidemiology of VTEC and cryptosporidiosis.

Methods

VTEC and cryptosporidiosis notifications, denominator and animal density data were obtained for the years 2004 to 2009. Notification rates of VTEC and cryptosporidiosis were described in relation to time, person, and rurality. The relationships between the density of each of the major farm animals in New Zealand and disease were evaluated using Poisson regression. Age, sex, ethnicity, deprivations and animal density were included as covariates. Notification data, animal density and denominator data were linked at the level of meshblocks.

Results

Notification rates of VTEC and cryptosporidiosis increased over the study period and varied by season. For both diseases, rates were highest amongst individuals less than five years old, those in the least deprived areas, and those of European ethnicity. For each animal type, rates were higher in areas with animals relative to those with no animals. Within dairying areas, relative to the lowest dairy density areas (between 0 and 25 animals/km^2 exclusive), adjusted rates of VTEC were significantly higher in some dairy areas but there was no linear gradient. In contrast, there was a linear gradient between dairy density and cryptosporidiosis (p for trend <0.001); a similar relationship was observed for cryptosporidiosis in sheep areas. There was a trend for decreasing VTEC and cryptosporidiosis rates as beef cattle density increased ($p<0.075$ for VTEC and $p<0.001$ for cryptosporidiosis).

Conclusion

The findings of this study, combined with those from other research, suggest a causal association between both dairy cattle density and sheep density, and cryptosporidiosis in New Zealand. While the association between dairy density and VTEC in this study was less clear, the higher rates of VTEC in some dairying areas warrant further attention. The findings of this study suggest that rural land use changes in New Zealand may have adverse effects on human health. In this context, this study recommends greater involvement of the public health sector in agricultural policy making, planning and research.

Introduction

The central aim is of this study is to assess the relationship between farm animal density and the incidence of Verocytotoxigenic *Escherichia Coli* (VTEC) and cryptosporidiosis in New Zealand. To supplement this central aim, the study also describes the epidemiology of VTEC and cryptosporidiosis. Chapter 1 provides a background to VTEC and cryptosporidiosis and the relationship with agricultural land use. Chapter 2 reviews the literature on the relationship between animal density and VTEC or cryptosporidiosis. Chapter 3 presents the objectives and methods used in the study and Chapter 4 presents the results. Finally, Chapter 5 discusses these results, outlines implications of the study, and recommends further research directions.

1 Background

1.1 Emerging infectious diseases

Emerging infectious diseases (EIDs) amongst humans can be defined as those "that have newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic range" (1).

An increase in EIDs has been demonstrated since 1940 independent of the effect of better reporting (2). Recent examples of EIDs include the Human immunodeficiency virus (HIV) and the Variant Creutzfeldt-Jacob disease (vCJD) (3).

While a broad range of factors contribute to the increase in EIDs, including socio-economic factors such as travel and trade (1-5), human-induced ecological disturbances are among the most frequently identified factors (5-7).

One form of ecological disturbance considered to be especially important in EIDs is agricultural land use development (3, 5-7). Such development includes encroachment of agriculture into forested environment as well as the intensification of agricultural animal farming.

Agricultural animals are important in the emergence of infectious diseases as they can act as a "zoonotic pool", or reservoir, from which previously unknown pathogens can emerge (8). Diseases caused by pathogens that are naturally transmitted between animals and humans are called zoonoses (9). Zoonoses are responsible for more than 60% of EIDs (2, 10).

Verocytotoxigenic *Escherichia coli* (VTEC) and cryptosporidiosis are two emerging zoonoses. Agricultural animals act as reservoirs for the pathogens that cause these diseases (11, 12). Theses pathogens, VTEC and *Cryptosporidium* cause significant disease within human populations (13, 14). They can be transmitted from animals to humans via direct contact, or indirectly though contact with contaminated water or food (14, 15).

This background describes the emergence, classification and clinical features of VTEC and cryptosporidiosis. The role of agricultural animals as reservoirs of these diseases is discussed, and transmission pathways related to agricultural land use are outlined. Finally, VTEC, cryptosporidiosis and land use in a New Zealand context is discussed

1.2 VTEC and cryptosporidiosis

1.2.1 International emergence

VTEC was first described as a human pathogen in 1983 in the United States after an outbreak of gastrointestinal illness, including haemorrhagic colitis, was found to be associated with ground beef "fast food" (16). VTEC has subsequently become an important emerging zoonotic infection worldwide (17), and is associated with significant morbidity in many countries including Canada (18), Scotland (19) and New Zealand (20). Since the emergence of VTEC, foodborne transmission has been a significant research focus; as well, transmission pathways related to agricultural land use are considered increasingly important (17, 21).

Cryptosporidium was first described as causing human disease in 1976 (22). However, it was only with the emergence of the acquired immunodeficiency syndrome in 1982 that it became a prominent human pathogen (23). Since then, cryptosporidiosis has developed into a common cause of gastroenteritis worldwide amongst immunocompetent humans (14). One large study in developed and developing countries showed the prevalence of cryptosporidiosis in people with diarrhoea was between 2 and 6% (24). Within children of developing countries this prevalence is even higher. Studies in India, South Africa, Uganda, and Brazil have identified *Cryptosporidium* in 15%, 18%, 25% and 19% of childhood cases of diarrhoea

respectively (25). In terms of maintaining the incidence of cryptosporidiosis, the World Health Organisation recognises land use as an important factor, and state that one of the primary drivers of cryptosporidiosis is "poor watershed management where livestock exist" (26).

1.2.2 Classification of VTEC and *Cryptosporidium*

VTEC are *Escherichia Coli* bacteria that produce verocytotoxins. Because these toxins are similar to Shiga toxin produced by *Shigella dysenteriae*, the term VTEC is interchangeable with STEC (Shiga-toxin producing *E. coli*). There are approximately 250 different serogroups of VTEC (27) and at least 100 are pathogenic to humans (27). The serogroup 0157:[H7], hereafter referred to as 0157, is the most widely recognised to be associated with human disease and arguably the most virulent (13, 15). However, non-0157 serogroups have recently been recognised as being important pathogens (27), and in some countries non-0157 VTEC notifications outnumber 0157 notifications (28).

Cryptosporidium is the genus name of a group of at least 20 species of protozoa. (25), whose classification has been frequently revised (29). *C. parvum* (genotype 2: "bovine" genotype) and *C. hominis* are the two *Cryptosporidium* species that are most important in causing human disease (25, 29). *C. hominis*, known as *C.parvum* genotype 1 ("human" genotype) prior to 2002, largely infects human population and rarely infects other species (30). By contrast, *C. parvum* genotype 2, the "bovine" genotype, is known so far to infect mainly ruminants and humans (29).

1.2.3 Clinical features

1.2.3.1 VTEC

After an incubation period of three to eight days (13), VTEC typically presents with diarrhoea and abdominal cramps. Bloody diarrhoea, associated with haemorrhagic colitis, is frequent and results from verocytotoxins damaging gastrointestinal endothelial cells (31). Fever is often absent which may trigger a clinician to look for a non-infectious cause of bloody diarrhoea such as intussusception or ischaemic colitis. VTEC infection can be diagnosed by culture of stools on Sorbitol-MacConkey agar plates. Many non-0157 serogroups will not be detected using culture methods alone and confirmation by PCR or immunoassays for Shiga toxins is required (13, 31).

In most cases, VTEC resolves in about six to eight days with no important sequelae (13). However, approximately two to seven percent of patients with VTEC will develop haemolytic uraemic syndome (HUS) (13), which can occur after verotoxins migrate into the systemic circulation (32). HUS is a serious, often life threatening illness comprising microangiopathic haemolytic anaemia, thrombocytopaenia and acute renal failure. Up to 96% (range 30-96%) of HUS cases are caused by VTEC (13). Children are at particularly high risk. A large meta-analysis of HUS in children showed the case fatality rate was 9%. For survivors, 25% had long-term renal sequelae such as proteinuria or renal insufficiency (33).

Treatment of diarrhoea caused by VTEC, as well as HUS, is supportive. Antibiotics are contraindicated as they do not improve clinical outcomes and, in the presence of VTEC, their use increases the risk of HUS in children (34).

Asymptomatic infection by VTEC occurs (13, 15) and is particularly well documented during outbreaks (15). Furthermore, VTEC shedding can continue for several weeks after the resolution of the illness (15). These findings suggest that humans may act as reservoirs of VTEC even while asymptomatic.

Most of the research into the clinical features of VTEC is based on the 0157 serogroup, and information on the clinical features of non-0157 serogroups is more limited. However, research suggests that the illness caused by some non-0157 serogroups may be equivalent to the severity of that caused by 0157 (27). Because of the large number and variability in non-0157 serogroups, the diversity of clinical features of these VTEC strains is likely to be greater than 0157 (27).

1.2.3.2 Cryptosporidiosis

Cryptosporidiosis usually presents with watery diarrhoea after the protozoa invades and multiplies in the gastrointestinal epithelium. The incubation period is approximately one week (range 1-30 days) (25). Other symptoms including abdominal cramps, nausea and vomiting are common (25). While diagnosis is usually by microscopic examination of the stool (25), this will not differentiate between diseases caused by *C. parvum* or *C. hominis*; to differentiate these species reliably, PCR genotyping is required (30).

The median duration of cryptosporidiosis is five to ten days (25), but while generally self-limited, the condition can persist well beyond this period. Amongst immunocompetent adults and children, up to 45% of infections persist beyond 14 days (25).

In immunodeficient populations, especially those with HIV infection, cryptosporidiosis persistence is more likely than in immunocompetent populations (14). In addition, symptoms of disease within immunodeficient groups are usually more severe, and can result in severe cachexia and even death (14).

Asymptomatic cryptosporidiosis infection can occur in healthy individuals (14, 35) and those who have had symptoms may continue to excrete oocytes up to eight weeks after symptoms have resolved (14). Therefore, such individuals may act as reservoirs of infection within human populations.

Like VTEC, treatment for cryptosporidiosis is supportive. Antimotility agents have been suggested to be an important part of therapy, but are only useful in mild disease (25). Trials have been conducted in immunodeficient populations investigating the effect of antiparasitic drugs such as nitazoxanide and macrolide antibiotics, but there is no clear evidence that they are efficacious (25).

1.2.4 Agricultural animals as reservoirs of VTEC and *Cryptosporidium*

Research into the reservoirs of VTEC has concentrated on the 0157 serotype, which is likely to be due to its significance as a human pathogen. For this serogroup, there are features of its ecology that are so consistent across countries that generalisations can be made. To begin with, VTEC is ubiquitous in cattle populations, and while shedding of bacteria occurs intermittently throughout the year, it can be found in cattle on virtually all farms (11). The point prevalence of 0157 in dairy and beef cattle in North and South America, Europe and Asia varies from less than 1% to almost 10% (13, 15). The higher rates are more likely to be in heifers and calves less than one year old. Very young animals (less than three months) tend to have lower infection rates (11, 15). Next, while VTEC infection is associated with symptoms in some animals, the vast majority of infections in animals are asymptomatic (36, 13). Finally, while research into the reservoirs of VTEC has concentrated mostly on cattle, other ruminants, including sheep (36) and goats (37), are also likely to be important reservoirs for the pathogen (17). While VTEC has been isolated from horses, dogs (15) and a variety of other animals such as pigs, rabbits, and possums (17), Molbok states that the significance of

these non-ruminants as reservoir species is unclear (17). This author suggests that such findings may be due to transient carriage or may indeed reflect a wider number of reservoir species.

While research into the ecology of non-0157 serogroups is more limited than for 0157, non-0157 ecology is likely to be similar with some exceptions. Notably, faecal prevalence in bovine species tends to be higher than the 0157 serogroup (2.9-67.4%), which is likely to be due to the larger number of serotypes (13).

Since humans are the main reservoirs host of *C. hominis,* the association between agricultural animals and cryptosporidiosis will therefore be related to *C. parvum.* Based on microscope studies, more than 150 species of mammals have been identified as hosts of *C. parvum* or *C. parvum*-like organisms (29, 38). However, with the use of more sensitive techniques, including molecular characterisation, *C. parvum* has been found to infect mainly ruminants (especially cattle, sheep and goats), and humans (29). Deer, mice, and pigs possibly act as minor hosts of *C. parvum* (29); cats and dogs are not thought to carry *C. parvum* (12). Symptomatic *C. parvum* infections in ruminants are mostly a problem in the neonatal period (mostly 1-2 weeks) (39). Infected animals can excrete up to 10^6-10^7 oocytes per gram of faeces (40) and contribute to significant economic losses for farmers (39). Cryptosporidiosis in neonatal animals may result in a degree of acquired immunity (41). Cryptosporidiosis infection (usually asymptomatic) can also occur in adults (14). However, adult infections are thought to less common than in young animals and are usually sporadic (42, 43). Therefore, the majority of *C. parvum* produced on farms is thought to be excreted by calves (or other young animals) (43).

The prevalence of *C. parvum* in ruminants reported in the literature varies significantly. The sporadic and asymptomatic nature of the majority of cryptosporidioisis makes it difficult to assess prevalence accurately. Furthermore, studies often do not report the *Cryptosporidium* serotype isolated (for example (44)). However, a 6-year UK longitudinal study of healthy ruminants identified the prevalence of *C. parvum* in adult animals, including sheep and beef, of between 0% and 25% and a generally higher prevalence (13% to 52%), for calves and lambs (42). These prevalence findings are broadly consistent with those from studies in different settings, including studies of dairy cattle in New Zealand (40, 45, 46).

1.2.5 Transmission of VTEC and *Cryptosporidium* between agricultural animals and humans

If farm animals are acting as reservoirs of VTEC or *Cryptosporidium*, waterways used by humans for recreation or drinking can be contaminated by these pathogens (17, 47, 48). Therefore, within farming areas, waterborne transmission of VTEC and *Cryptosporidium* is of particular concern. While contamination of water with pathogens such as VTEC or *Cryptosporidium* can occur through direct contact of animals to streams (49), it is thought to primarily occur through faecal runoff into surface water (50, 51). This runoff is increased by factors including heavy rainfall (52) and high densities of livestock farming (53). Thus, as the intensity of livestock farming increases, as it is in New Zealand (54), contamination of waterways by pathogens carried by agricultural animals would also be expected to increase.

Internationally, numerous outbreaks of VTEC have been linked to water contaminated by agricultural animals (17). For example, in 2000 in Walkerton, Canada, faeces from livestock, together with heavy rainfall and an overwhelmed water treatment facility were implicated in causing 2300 cases of gastroenteritis (due to both VTEC and *Campylobacter*), 27 cases of HUS and seven deaths (55). For cryptosporidiosis, while water contaminated by agricultural animal runoff has also been repeatedly implicated as contributing to outbreaks (25), few studies have investigated the genotype of the *Cryptosporidium* species that caused the outbreak (48). Of those that have, *C. hominis* is the species most frequently identified (25). For example, *C hominis* was the causative agent of the world's largest recorded waterborne outbreak in Milwaukee, USA (57) which caused an estimated 403,000 illnesses (56). Nevertheless, other investigations have identified *C. parvum* as the species responsible for some outbreaks (58). Therefore, both *C. hominis* and *C parvum* are likely to be important in waterborne transmission pathways.

The transmission of VTEC and *Cryptosporidium*, particularly for the waterborne route, is likely to be facilitated by several factors. Both organisms have a small infectious dose, estimated at 50 organisms for VTEC (59) and between 10-1000 oocytes for *Cryptosporidium* (14, 35). In addition, both organisms are capable of surviving a long time in the environment, particularly if moist. To illustrate, VTEC has been shown to survive for up to 70 days in bovine faeces (60) and *Cryptosporidium* oocytes can remain infectious for more than six months in moist conditions (38). Furthermore, while the organisms are different in the resistance to water treatment (for VTEC, chlorine is adequate (61) whereas for *Cryptosporidium*, ultra-violet water treatment or reverse osmosis filtration is required (38)),

for both organisms, in the presence of water contamination, at least some treatment is required. This may explain why waterborne outbreaks are frequently associated with water treatment failure for both VTEC (17) and *Cryptosporidium* (43).

Direct transmission between agricultural animals and humans has been demonstrated for both VTEC (62) and cryptosporidiosis (12), and is another key pathway which places those living in areas with agricultural areas at higher risk of disease.

Other transmission pathways are food-borne and person-to-person transmission. Food-borne transmission has been the most commonly investigated mode for VTEC (13, 15) and while less common, has also been demonstrated to occur for cryptosporidiosis (25). Person-to-person transmission of both VTEC (63) and cryptosporidiosis (64) also occurs; this transmission mode is most likely to be observed in hospital or pre-school settings (13, 25).

1.2.6 Agricultural animal farming in New Zealand

New Zealand is a country of approximately 4.4 million people and is highly agriculturalised. From an economic standpoint, excluding forestry, approximately 40% of New Zealand export earnings are currently derived from agriculture (65). The dairy industry is New Zealand's single largest export earner, generating half of these earnings (over 20% of total exports). The second largest earner is the meat industry which trading meat and edible offal, generates 13% of total export earnings (66).

In order to generate such incomes, it is understandable that there are large numbers of agricultural animals in New Zealand. The dominant species are sheep and cattle. In 2009, there were approximately 38 million sheep, six million dairy cattle, and four million beef cattle (67). As indicated above, these animals can carry VTEC and *Cryptosporidium*. Therefore, they are a large potential reservoir for these pathogens. While the degree to which these animals are acting as reservoirs of VTEC and *Cryptosporidium* within New Zealand is unknown, studies have confirmed that at least some New Zealand sheep, beef or dairy cattle carry VTEC (68, 69) or *Cryptosporidium* (40, 45, 70, 71).

Like other countries in the world, a concerning consequence of such large numbers of agricultural animals in New Zealand is microbial pollution of New Zealand's freshwater (72, 73). Agriculture has been implicated as the largest polluter of freshwater in New Zealand (49), and there is evidence showing the microbial water quality (as measured by indicator

bacteria) is worst where land use is most intensive (53, 72, 74). These trends are concerning as microbial pollution of freshwater may also relate to VTEC or *Cryptosporidium* contamination of waterways used for drinking or recreation by human populations. While there is no data on the extent of water contamination by VTEC in New Zealand, *Cryptosporidium* contamination of New Zealand's is widespread for surface water (75, 76). Furthermore, a significant proportion of New Zealand's population is served by water supplies that are not compliant with the *E. coli* and protozoal drinking water standards of New Zealand (at least 9% and 26%, respectively) (77).

1.2.7 VTEC and cryptosporidiosis in New Zealand

For VTEC within New Zealand, the annual notified incidence rate has risen dramatically since the first described case in 1993 (78) and in 2008, three cases per 100,000 were notified (79). This rate is high relative to notification rates in other developed countries including the United States, the United Kingdom, Germany, France, and Australia (79).

Within New Zealand, the majority (95%) of VTEC notified is identified as serotype 0157 (79). However, the 0157 serotype is more likely to be tested for by laboratories (79). Thus, it is possible that non-0157 serogroups go undetected and make up more than 5% of all VTEC cases.

For cryptosporidiosis within New Zealand, notification rates are approximately 22 cases per 100,000 per year (1997-2006 average annual rate) and like VTEC, are also high relative to those of the developed countries listed above (80). Currently within New Zealand, *C. parvum* and *C. hominis* are not differentiated for notification purposes (81). While research conducted by Learmouth et al. suggests that there are approximately equal numbers of the two species notified across New Zealand (70), *C. parvum* predominates in rural areas and is by far the dominant species in spring (45, 70, 82).

For both VTEC and cryptosporidiosis (all species of *Cryptosporidium*), notification rates are higher in rural than in urban areas (79, 83). Given agricultural animals are the major reservoirs of the diseases, this urban-rural gradient suggests direct transmission from contact with animals and contaminated environments or indirect transmission from rural water supplies (79). This urban-rural gradient has been observed internationally for both VTEC (84, 85), and cryptosporidiosis (86), and has stimulated researchers to assess whether or not the density of major reservoir species (in particular, agricultural animals) influences the rates of

disease in the areas from which the cases are notified. The literature detailing such analyses will be reviewed in more detail in the Literature review section.

Within New Zealand, there have only been preliminary investigations of the influence of agricultural animal density on disease rates. The study by Snel and Baker revealed that farm animal density was positively correlated with cryptosporidiosis (80). While this study indicated that both cattle and sheep may be important (80), methodological limitations meant that the effect of different animal species was not able to be determined.

To extend on research to date, the central aim of the present study was to identify the relationship between the density of the each of the major farm animals in New Zealand (dairy cattle, beef cattle and sheep) and the rates of notified VTEC and cryptosporidiosis. To supplement and support the central aim, the study also described the epidemiology of VTEC and cryptosporidiosis.

2 Literature review

2.1 Objectives

This review will:

1. Describe recent studies that have examined the spatial relationship between cattle or sheep, and VTEC or cryptosporidiosis rates in human populations
2. Identify key factors that may influence the relationship between ruminant animal density and VTEC or cryptosporidiosis from the described studies

2.2 Methods

The peer-reviewed literature on the association between ruminant density and human enteric disease was searched. The search terms used were (all subject heading unless specified): 1) Ruminants, cattle, sheep combined with the Boolean operator OR; 2) Gastroenteritis, Shiga-Toxigenic *Escherichia Coli*, cryptosporidiosis, combined with OR; 3) Population density and the keywords (with wild card) intensif$ and densit$ combined with OR. These three search terms were then combined with the Boolean operator AND. Using the Medline database from 1950 onwards, 69 articles were obtained, nine of which were directly applicable to the objectives of this paper. One additional article was found by searching the Embase database. By searching the reference lists of retrieved papers and by key authors, two other articles were retrieved, bringing that total to 12 articles.

Table 1 provides an overview of each study showing the year published, location, main exposure and outcomes assessed; a summary of the methods used, and the most relevant results.

2.3 Literature review commentary

2.3.1 Introduction

This review will provide an overview of studies found and the reasons for the relatively recent development of work in this area. It then reviews the most relevant New Zealand and overseas studies. Some inherent problems with the studies reviewed and other relevant general

methodological issues will be discussed, before presenting conclusions made from this review.

2.3.2 Overview of outcome measures and study locations

Twelve studies, all of ecological study design, were identified having investigated the relationship between ruminant density and VTEC or cryptosporidiosis (see Table 1). The majority (7/12) focused on VTEC (18, 19, 28, 85, 87-89); one study was on haemolytic uraemic syndrome (HUS) in children (usually caused by VTEC infection) (90); two on cryptosporidiosis (83, 86); and two on 'Gastroenteritis', including either cryptosporidiosis (91) or VTEC (92). All 12 were performed in developed countries, including two in New Zealand. Of the remaining 10 studies, four were undertaken in Canada (18, 85, 87, 92), two in Scotland (19, 89), and one each in France (90), Germany (28) and Sweden (88).

There were no studies on this subject before 1999. This relatively late development is not only because VTEC and cryptosporidiosis were not identified as human pathogens until 1983 (16) and 1976 (22) respectively, but also because such spatial ecological studies need quality geographical data on environmental risk factors, human health outcomes, and human populations (93). Until relatively recently, such data were difficult to obtain (93). In addition, in the last 10 years, there has been increasing awareness and attention given to the role agricultural land use (including cattle and sheep farming) has on the development and emergence of infectious diseases worldwide (5, 26), hence increasing research interest in this area.

2.3.3 New Zealand Studies

Snel et al. (83) in 2009, examined the relationship between the density of "farm animals" (cattle, sheep, deer and horses combined), and rates of cryptosporidiosis in New Zealand. While they identified a significant positive relationship between these, the study was not specific enough to examine the role of different species of ruminants. In addition, potential confounders such as age, and ethnicity were not controlled for and the geographical unit used in the study was large (territorial authority,) which made the analyses more vulnerable to ecological bias than if smaller units were used.

Close et al. (91) in 2008, compared age-standardised rates of specific notified enteric diseases, including cryptosporidiosis, in "dairying areas with major irrigation" (mostly flood irrigation) within a small area in South Canterbury, with those in the "rest of Canterbury". They also

compared rates of disease in the major irrigation areas with those in "dairying areas without major irrigation". The cryptosporidiosis rate in the major irrigation areas was 5.3 times higher than the rest of Canterbury, and 2.1 times than the areas without major irrigation. As this study did not measure ruminant density, no assessment of the relationship between levels of dairy cattle density and rates of cryptosporidiosis could be undertaken. Nevertheless, the study's results suggest that the density of dairy cattle may be an important factor in determining cryptosporidiosis rates, assuming that areas with major irrigation carried a higher density of dairy cattle than areas without. An alternative explanation is that the higher rates in areas with major irrigation may be related to greater microbial contamination of groundwater beneath the areas with major irrigation, independent of dairy density. Specific limitations of this study were that the 'exposed' population was small (5,088) and from one area in Canterbury, thus differences in rates could be attributed to biases in disease testing and/or notification rates. Such bias could exist if a local physician serving the dairying areas tests and/or notifies disease more than his/her equivalent in other areas.

No New Zealand studies have examined the relationship between ruminant density and VTEC.

2.3.4 International studies

2.3.4.1 Exposure measures reported

Of the 10 international studies reviewed, while all had at least one measure of ruminant density as an exposure measure, some assessed other indirect measures. For example, Kistemann (88) used "prevalence of positive [VTEC] samples [from] abattoirs", and Valcour (87) the "proportion in a CCS [area] having manure applied to land via a solid spreader". Some of the studies only reported the relationships between exposures and disease for their best fitting models Therefore, some studies with multivariate analyses (for example (86, 88)) did not report on the effect of ruminant density on disease rates in their final analyses.

2.3.4.2 Studies examining the relationship between cattle and VTEC or cryptosporidiosis

In Ontario, Canada, Michel (85) found a positive and significant linear association between total cattle density, total livestock density and age standardized sporadic VTEC rates. The size of the cattle density effect could not be calculated from the data provided. Despite the large number of cases in the study (n=3001), and dairy and beef cattle being separately analysed, dairy cattle density was not reported to be significantly associated with VTEC rates.

A study by Valcour et al. (87) from the same Canadian research group, analysed a large number (80) of agricultural exposure variables in multivariate models with crude sporadic VTEC rates as the outcome. The model that best predicted VTEC rates included as one of the four covariates in the model, the 'ratio of beef cattle (not total or dairy cattle) to human population'. While highly statistically significant, the effect size was not given.

Taken together, these two Ontario studies suggest that in Ontario, beef cattle (either density or ratio with human population) predict VTEC rates better than dairy cattle density alone.

These findings contrast with those of Haus-Cheymol et al. in France (90). They found that in the multivariate analyses (adjusting for rural degree, latitude and longitiude, temperature and precipitation) only dairy cattle, (and not beef cattle), was an important predictor. While this study used HUS, not VTEC, as the outcome, it is likely that HUS incidence can act as a surrogate for VTEC infection, as up to 96% of HUS cases are caused by VTEC infection (13). They suggested several possible reasons why their findings differed from those in Ontario, and in particular because of differences in the size of the dairy industry, or manure management practices.

As discussed below, of the remaining six international studies reporting the relationship between one type of cattle density in the final analyses, the majority (four out of six) reported a positive association between at least one measure of cattle density and VTEC or cryptosporidiosis (18, 19, 28, 89). However, for some of these, the positive association was only in selected areas (19), or was dependent on which ruminant density measure was assessed (19), or whether sporadic or total VTEC cases were included (84)

Innocent et al. found that total cattle density was a significant risk factor for VTEC in an analysis that included all of Scotland, but not in an analysis excluding the south east of the country, an area of high cattle density. This highlights the influence one area might have over the entire analyses, suggesting there may be a threshold of cattle density that needs to be exceeded before the risk of VTEC within an area increases. This study included the variable "cattle per head of human population" in the final model, which was more highly significant than the measure "cattle density" in the same model. This "cattle per head of human population" measure was significant when the high-density cattle area was excluded, unlike "cattle density". The difference may have been that "cattle per head of human population"

was a more sensitive estimator of human exposure to VTEC in cattle faeces than “cattle density”.

Pearl et al. performed a study in Alberta, Canada (84) and was the only one reviewed that analysed total and sporadic cases separately. They found a consistent positive association between the highest quartile of total cattle density and total VTEC rates, but not when limited to sporadic cases. Such a finding suggests that outbreak cases (included in total cases), are more closely associated with cattle density compared with sporadic cases. However, these findings contrast with the other Canadian studies which had only sporadic cases as an outcome and found a positive association with cattle density measures.

Two reviewed studies did not find a positive association between cattle density and VTEC (92) or cryptosporidiosis (86) in their final model. The study by Febriani et al. in Quebec, Canada had zoonotic gastroenteritis as an outcome, and like Haus-Cheymol et al., analysed hospitalised cases. Notification bias is less likely to be a problem using hospitalised cases as the presentation of those with severe disease to medical services is less likely to be influenced by factors that may be associated with exposure. However, while there were 526 cases of zoonotic gastroenteritis overall, only 90 cases of *E. coli* were available for separate analyses, and no cryptosporidiosis cases. It is possible that such low numbers of *E. coli* cases may have contributed to this study being insufficiently powered to detect an association between either beef or dairy cattle and *E. coli* gastroenteritis.

Pollock et al. (86) conducted a study in Scotland with a relatively small number of cases of cryptosporidiosis caused by *C. parvum* (n=284) and *C. hominis* (n=276). They suggested that *C. parvum*, having a zoonotic reservoir and similar transmission pathways to VTEC, may show the same spatial distribution as VTEC Scotland as was demonstrated by Innocent et al. (19). On the other hand, *C. hominis*, being epidemiologically associated with humans, was expected to show a different spatial distribution less influenced by farming related variables. Cases caused by *C. parvum* and *C. hominis* were analysed separately in a multivariate Poisson model then compared in negative binomial model. In the multivariate Poisson model, cattle density was not included as there was no univariate association between cattle density and *C. parvum*, an unexpected finding. However, in the negative binomial model, as predicted, as cattle density increased *C. parvum* was more likely to be notified compared to *C. hominis.* One possible explanation for the unexpected findings of the Poisson model may have been that the association between cattle density and *C. parvum* was not obvious in the univariate

model, but may have become significant in the multivariate model. Alternatively, the numbers of *C. parvum* cases may have been too small to detect a significant univariate association.

2.3.4.3 Association between sheep and VTEC or cryptosporidiosis

Only two studies examined the relationship between sheep density and VTEC. In Ontario, Valcour et al. (87). did not identify a significant association between sheep density and VTEC infection; they commented that this may have been due to the relatively low numbers of these animals compared to other animals in the study area. In contrast, Strachan et al. (89) in the Grampian area of Scotland, found that the increasing ratio of "sheep density/human population density" significantly predicted increasing rates of VTEC.

Only one study examined the relationship between sheep density and cryptosporidiosis. Pollock et al. (86) found a significant positive relationship between sheep density and cryptosporidiosis in Scotland. They also identified that as sheep density increased, cryptosporidiosis due to *C. parvum* was more likely to be notified when compared with that due to *C. hominis*. This finding is consistent with sheep being an important reservoir of *C. parvum* (94).

2.4 Publication and methodological issues relevant to reviewed studies

While the results reviewed may provide some insights into the association between cattle or sheep density and VTEC or cryptosporidiosis, there are several issues to consider before making conclusions from them.

One issue is related to research focus and which results are presented for publication. Most of the research has focused on the association between cattle density and VTEC or cryptosporidiosis and paid relatively little attention to other animals. For VTEC, this bovine focus reflects other research related to this pathogen (11, 13, 15, 17, 21) and occurs in spite of evidence showing other ruminants (such as sheep) may also act as important reservoirs of the pathogen (36, 37). As a result of this bovine research focus, no clear conclusions can be made about the importance of the densities of non-cattle animal species in terms of determining risk of VTEC in humans. Another related issue is that some of the papers only reported findings of their best fitting model. For example Kistemann et al. did not report cattle density in the final model, so there was no way of knowing the effect this variable had on the exposure of VTEC rates.

Another concern is related to methodological issues that could impact on the internal validity of the studies reviewed. Regarding power, it is noteworthy that all studies except those that were small, demonstrated a positive association between cattle density and VTEC or cryptosporidiosis. This indicates that the lack of consistency in the association between cattle density and disease may in fact be due to random error in small studies, rather than reflecting an absence of association. Also important is the collinearity between variables in statistical models that was shown to be present in those studies where it was examined and reported (88, 90). This is a common problem for spatial ecological studies, inasmuch as it may be difficult to separate out the effects of related variables in the statistical analyses (95). Thus, while some of the studies indicated that one type of cattle was more important than another in determining disease risk (85, 90, 96), the findings may not be reliable. Another issue is the control of confounders, factors that are associated with both the exposure (animal density) and outcome (disease), and not on the causal pathway. Some of the reviewed studies either attempted to adjust for agricultural or geographical confounders only (86, 87, 88, 97) or did not adjust for potential confounders at all (89, 83). Nevertheless, the conclusions drawn by the studies that did adjust for age either in models (18, 85), age standardization (91), or by stratification or restriction (28, 90), were similar to those that did not. Any differences could be explained by other factors such as differences in random error. A final methodological issue that needs to be considered is that of adjusting for spatial effects. These effects include clustering of cases and variability of disease rates that is greater than expected based on the assumptions of a simple statistical model (e.g. Poisson or linear regression). The significance of not accounting for spatial effects is that calculated standard errors and associated p-values may suggest a statistically significant result when this may not be the case. This is particularly relevant for those studies that had p values >0.01 (for example, that of Haus Cheymol et al.).

Also of relevance is the area and country in which research is carried out. As highlighted by Haus-Cheymol et al. and Valcour et al., variations in factors such as numbers of reservoir species and livestock management practices between areas may explain differences in results of the studies. Such factors can limit the external validity of the reviewed papers.

2.5 Summary

In summary, there have only been twelve studies that have investigated the relationship between cattle or sheep density and rates of VTEC or cryptosporidiosis. All have been performed in developed countries, including two in New Zealand. Both of the New Zealand studies had cryptosporidiosis as the main outcome, which is in contrast to the majority of international studies where VTEC was the focus. Most of the studies assessed the role of cattle as determinants of disease, and only three included sheep.

Generalisations about the meanings of the studies in terms of the relationship between cattle or sheep density and VTEC or cryptosporidiosis should be made with caution. Reasons for this include the small number of studies included, and methodological issues that limit the internal validity of the studies. Nevertheless, this review suggests that cattle density may be an important determinant of VTEC and cryptosporidiosis risk. While there were insufficient studies to indicate the role of sheep density on disease risk, preliminary evidence included in this review suggests that sheep may also be important in determining VTEC and cryptosporidiosis risk. The review also showed that the results of studies varied by factors specific to a country or areas. Thus, in order to examine the association between cattle or sheep density and VTEC or cryptosporidiosis in New Zealand, New Zealand-specific analyses are required.

Table 1: Table of twelve ecological studies that have examined the relationship between ruminant density (or a surrogate) and VTEC or cryptosporidiosis (or a surrogate).

Study authors, publication year and location	**Outcome (whether sporadic or total cases included)**	**Main exposure variables assessed (including potential confounders)**	**Methods**	**Results, including effect size and p-value (where available)**
Michel, et al., 1999 Ontario, Canada (85).	VTEC (sporadic). 3001 cases.	Total cattle density, dairy cattle density; total livestock density other than cattle and total livestock density.	Multivariate linear regression models.	Positive association with cattle density ($p<0.001$) and total livestock density ($p=0.002$). Effect size not able to be calculated from data.
Valcour et al., 2002 Ontario, Canada (96).	VTEC (sporadic), 1,275 cases.	Multiple (80) livestock density indicators assessed including: density of beef cattle, total cattle, sheep, and total livestock and ratio of beef cattle to human population.	Multivariate Poisson regression models.	Strongest associations with beef cattle per human population ($p<0.001$). No association between sheep and VTEC incidence. Effect size not able to be calculated from data.
Kistemann et al., 2004, Sweden (88).	VTEC (total). 525 cases.	Cattle density; prevalence of VTEC infection in cattle.	Univariate and multivariate linear regression models.	Cattle density correlated with VTEC infection (Pearson's coefficient: $R^2=0.55$, $p<0.001$). Best fitting multivariate model: prevalence of positive VTEC samples at abattoirs associated with VTEC incidence ($p=0.0001$).
Innocent et al., 2005, Scotland (97).	VTEC (sporadic). Numbers not reported.	Cattle population density; cattle per head of human population; human population density; latitude; longitude.	Multivariate Poisson regression models.	"Cattle per head of human population" significant ($p<0.001$). Cattle density significant for all Scotland ($p=0.021$) but not when high density cattle area excluded ($p=0.274$).

Study authors, publication year and location	Outcome (whether sporadic or total cases included)	Main exposure variables assessed (including potential confounders)	Methods	Results, including effect size and p-value (where available)
Frank et al., 2008, Germany (28).	VTEC (total). Non non-0157 serotypes dominant. 3216 cases.	Total cattle density; age of case.	Bayesian Poisson regression models. Stratification by age.	Every increase in 100 total cattle per sq km led to 68% increase in VTEC risk (95% credible interval 1.11-2.5). Risk of VTEC higher in younger age groups (37 fold increased risk for those <2 years old compared with >10 years old).
Strachan et al., 2006, Grampian, Scotland (89).	VTEC (not-specified) 392 cases.	Cattle density per human population. Sheep density per human population.	Linear univariate regression models.	Positive association for cattle density per human population ($p<0.001$). Positive association for sheep density per human population ($p=0.001$).
Haus-Cheymol et al., 2006, France (90).	Haemolytic uraemic syndrome (as a surrogate for VTEC infection) in children <15 years. Person to person transmission excluded. 451 cases.	Total cattle density; dairy cattle density; beef cattle density; calf density; total adult cattle density; ratio of calves per population of children less than 1 years old (all in quartiles).	Univariate and multivariate Poisson regression models.	In multivariate model, dairy cattle (but not beef cattle) associated with HUS ($p<0.02$ for all quartiles). RR range from 1.39 (for cattle density 0.11-0.29/ha vs 0-0.04/ha) to 1.85 (for 0.29-0.46/ha vs 0-0.04/ha. Ratio of calves per children associated with HUS ($p<0.03$ for all quartiles). No clear dose response for either variable.

Study authors, publication year and location	**Outcome (whether sporadic or total cases included)**	**Main exposure variables assessed (including potential confounders)**	**Methods**	**Results, including effect size and p-value (where available)**
Pearl et al., 2009, Alberta, Canada (18).	VTEC (sporadic and total cases analysed separately). 869 total cases, 684 sporadic cases.	Total Cattle density (quartiles); aboriginal identity; percentage of movers; percentage of low-income households.	Multivariate Poisson and negative binomial regression models.	Best fitting model showed significant relationship between cattle density quartiles and total cases (Q4 vs Q1 RR 2.40 (95% CI: 1.16-4.98)) but no clear dose response. No significant relationship between cattle density and sporadic VTEC cases in best fitting model.
Febriani et al., 2009, Quebec, Canada (92).	Acute gastroenteritis hospitalisations including *E. coli* 90 *E. coli* cases.	Dairy cattle, beef cattle, swine, poultry, and combined total animal density; manure surplus; water source; age; sex, and deprivation index.	Multivariate Poisson regression models.	Multiple models fitted. No association between dairy cattle or beef cattle and gastroenteritis due to *E. coli*, but association between swine density and gastroenteritis due to *E. coli.*
Pollock et al., 2009, Scotland (86).	*Cryptosporidium parvum* (sporadic): 276 cases. *C. hominis* (sporadic): 284 cases.	Cattle, sheep, deer, pig, goat and human population density; ratio of farms to human inhabitants; latitudinal and longitude; private water supply/ human inhabitants.	Multivariate Poisson regression models. Binomial models for species specific comparison.	Increased risk of *C. parvum* with: increased sheep density, decreased human population density, increased farms per person, increased private water supply per person and the further east a district was located (all $p<0.001$). Cattle density not included in final model. In binomial model, *C. parvum* more likely than *C. hominis* to increase as cattle and sheep density increase ($p<0.001$).

Study authors, publication year and location	Outcome (whether sporadic or total cases included)	Main exposure variables assessed (including potential confounders)	Methods	Results, including effect size and p-value (where available)
Close et al., 2008. Canterbury, New Zealand (91).	Enteric diseases including cryptosporidiosis (total). Number of cases not reported.	Dairy farms under "major irrigation schemes"; dairy farms without major irrigation; deprivation index (NZDep01).	Crude and age standardised rates for cryptosporidiosis.	Increased risk of cryptosporidiosis in dairy farms with major irrigation schemes relative to rest of Canterbury (RR & 95% CI: 5.33, 4.12-6.90). Relationship unlikely to be due to confounding by deprivation.
Snel et al., 2009, New Zealand (83).	Cryptosporidiosis (total). 821 cases.	Farm animal (cattle sheep deer and horses) density.	Linear regression model.	Positive linear correlation between total farm animal density and cryptosporidiosis. Effect size not calculated.

3 Methods

As mentioned at the end of the Background section, the central aim of this study was to identify the relationship between the density of each of the major farm animals in New Zealand (dairy cattle, beef cattle and sheep), and the rates of notified VTEC and cryptosporidiosis. To supplement and support the central aim, the study also aimed to describe the epidemiology of VTEC and cryptosporidiosis in New Zealand.

This section begins by listing the objectives necessary to fulfil these aims. Following this, details on ethical approval, study area, population, and data sources are provided. It concludes by describing the statistical methods used to address the objectives.

3.1 Objectives

1. To describe the epidemiology of domestically acquired notified cases of VTEC and cryptosporidiosis in New Zealand from 2004 to 2009 in relation to:
    a. Time (year and season)
    b. Person (age, sex, ethnicity and deprivation index)
    c. Place (urban/rural)
2. To describe measures of dairy cattle, beef cattle and sheep density from 2004-2009 using data from the Agribase^{TM} database.
3. To perform preliminary analyses to identify the relationship between dairy cattle, beef cattle, sheep density, and the rates of domestically acquired notified VTEC and/or cryptosporidiosis within mainland New Zealand in the years 2004-2009.

3.2 Ethical approval

This study was approved by the Multi-region Ethics Committee of the Ministry of Health on 16 December 2009.

3.3 Study type, area and population

This study occurred in two main stages. The first stage was a descriptive analysis of both the human disease data and animal data, respectively (corresponding to objectives 1 and 2 above).

The second stage was a series of ecological analyses that examined the relationship between animal density and disease (corresponding to objective 3 above). For the ecological analyses, the meshblock[1] was the geographical unit.

Each meshblock has a unique identifier allowing linkage of VTEC or cryptosporidiosis notification case data, animal data and denominator data. As the boundaries of some of these meshblocks change at each census, the 2006 meshblock pattern was used. In 2006, there were 41,392 meshblocks in New Zealand which Statistics New Zealand has grouped into six categories, the vast majority (97.8%) referred to as "Mainland". The remainder were "Oceanic", "Island" (including Rakiura), "Inland waters", "Inlet" and "Other".

For this study only "Mainland" meshblocks were included. The reason for this was that other meshblock categories were either unlikely to have animals or people, or may have farming practices significantly different from mainland meshblocks. Of the mainland meshblocks, 1,723 were excluded because in the 2006 census they had a human population of zero, and as a consequence, it was not possible to calculate disease rates. Therefore, 38,743 meshblocks were included in the analyses (see results). The area of these varied from 0.05 hectare (1 hectare = 0.01km^2) to nearly 2000km^2. The median meshblock size was 0.077km^2. For illustrative purposes, if these meshblocks were square, the length of the boundary would be 22.5m for the smallest, 43.5km for the largest, and 256m. for the median. The human population of the meshblocks in the study varied from 3 to 1431, with a median of 93. Meshblock populations are randomly rounded to base three to preserve confidentiality.

The study population consisted of all people living in mainland meshblocks at the 2006 census (n=4,014,801).

[1]"The meshblock is the smallest geographic unit for which statistical data is collected and processed by Statistics New Zealand. A meshblock is a defined geographic area, varying in size from part of a city block to large areas of rural land. Each meshblock abuts against another to form a network covering all of New Zealand including coasts and inlets, and extending out to the two hundred mile economic zone. Meshblocks are added together to 'build up' larger geographic areas such as area units and urban areas. They are also the principal unit used to draw-up and define electoral district and local authority boundaries." (98)

3.4 Data sources

3.4.1 VTEC and cryptosporidiosis notifications

Cases of VTEC and cryptosporidiosis notified to the Institute of Environmental Science and Research (ESR) between 1 January 2004 and 31 December 2009 were used for both the descriptive and ecological analyses. This time period was chosen as agricultural data mapped to the 2006 meshblock pattern was only available for the years 2004-2009 inclusive.

The case definitions required a clinically compatible illness with corresponding laboratory confirmation. For VTEC, laboratory confirmation was through either detection of Shiga toxin-producing *E. coli* by use of selective culturing media, or by PCR detection of Shiga toxin-producing *E. coli*. However, within New Zealand most diagnostic labs only have the capacity to detect 0157 strains (through culturing); thus, 0157 serotypes are preferentially reported (79). For cryptosporidiosis, confirmation was theoretically through isolation of *C. parvum* oocytes from a faecal specimen (99), although in reality *Cryptosporidium* species were unlikely to be differentiated (81). Thus, the present study's analyses are likely to include cases of cryptosporidiosis caused by both *C. hominis* and *C. parvum*.

Both sporadic and outbreak cases of VTEC and cryptosporidiosis were included because it was not possible to accurately differentiate between them based on the information provided by ESR (100).

Those cases that had a documented history of overseas travel within the incubation period of the illness (defined as 2-8 days for VTEC and 1-12 days for cryptosporidiosis) were excluded, as the objective of this study was to examine the effect of dairy cattle, beef cattle or sheep exposures that occurred within the New Zealand environment.

Case information, detailing the meshblock of the usual residence of each case, age group, sex, ethnicity, deprivation and rurality were provided by ESR.

Ethnicity data was based on self-reported ethnicity data collected by the notifying agent (usually Public Health units). The Ministry of Health's Prioritised Ethnicity system was used (101). In this system, if multiple ethnicities are provided, each individual can have only one recorded. Māori, Pacific, Asian and 'Other' are respectively prioritised over the majority ethnicity, European.

The deprivation level of each meshblock was defined using the NZDep06 deprivation index. Briefly, this index combines nine socio-economic variables (income (two variables), employment, communication, transport, social support, qualifications, living space, and home ownership) in the 2006 census to give each meshblock a deprivation score. NZDep06 deprivation level 1 represents the least deprived, and level 10 the most deprived population. Further details on the NZDep06 index are provided by Salmond and Crampton (102)

Rurality was defined using Statistics New Zealand "Urban/Rural Profile" classification system (103). Urban and rural meshblocks are those within historically defined areas that have respective populations of 1000 or more, and 999 or less. Urban and rural meshblocks are further subdivided by Statistics New Zealand based on comparing usual residence address with workplace address (103). This comparison provides a proxy measure for the dependence a population within a given meshblock has on an urban area.

Urban meshblocks have been allocated to one of three categories, based on their dependence on "Main Urban areas" (103). "Main Urban areas" are those that have a population of >30,000, and include 12 centres in New Zealand in 2006 (e.g. Auckland and Dunedin). "Satellite Urban areas" and "Independent Urban areas" are those areas outside main urban areas where more than 20 percent or less than 20 percent, respectively, of the usually resident employed population's workplace address is in a main urban area (103). An example of the former is Rolleston, outside Christchurch, and of the latter is Westport, on the West Coast of the South Island.

Rural meshblocks have also been allocated to one of four categories based on their dependence on urban areas. These are 1) "Rural areas with high urban influence", 2) "Rural areas with moderate urban influence", 3) "Rural areas with low urban influence", and 4) "Highly Rural/Remote areas". Urban dependence progressively decreases for these categories. Unlike urban meshblocks, it is not possible to define rural areas based solely on percentage of population working in urban areas, as the weighting given to rural areas to define 'urban dependence' varies according to the type of urban areas the rural area is dependent on (103).

3.4.2 Numbers of dairy cattle, beef cattle and sheep

Numbers of dairy cattle, beef cattle and sheep for each meshblock in New Zealand were obtained from the Agribase^{TM} database. This database is maintained by AsureQuality, a New

Zealand government funded agricultural company (104). Agribase™ is a voluntary system developed in 1993 that provides a central index of farm types, farm use, ownership, location and management in New Zealand. Data on farm animals is collected throughout each year from farmers through various methods, including farm visits for Tuberculosis (TB) testing, or through rural rescue helicopter trust mailouts (105). Agribase™ database covers 96% of pastoral *lands* (grass land cover) when compared with Land Cover Database II held by the Ministry of the Environment (106). This coverage suggests Agribase™ is likely to be relatively complete in terms of recording animal numbers. However, it is not possible to be completely certain of the animal numbers as the overall percentage of New Zealand *farms* included in the database is unknown (105). The Agribase™ database is updated on a rolling basis. As of September, 46.5% of the farms in the data base were updated in the past 12 months, 21.5% in the previous 1 and 2 years, 17.3% in the previous 2-3 years, and 14.6% more than three years previously (105).

For this study, all animal data were mapped to the 2006 meshblock pattern by Agribase™ staff. Animal data for each farm were mapped to a meshblock according to the location of the main farm gate or homestead. If a farm spanned over two or more meshblocks, this was ignored (107).

For this research, dairy cattle included milking cows, replacement heifers, breeding bulls and calves. Beef cattle included beef cows, bulls, heifers and calves; and sheep included ewes, rams and lambs.

The land area (in km^2) used to calculate animal density, was based on the total land area of the meshblock. These data were provided by Statistics New Zealand.

3.4.3 Denominator data

All denominator data were obtained from Statistics New Zealand except for deprivation denominator data which were obtained from the Ministry of Health (108).

3.5 Statistical methods

The majority of the statistical analyses were conducted using Stata, version 11.0 for windows. Microsoft Excel was used for calculation of crude incidence rate ratios.

3.5.1 Descriptive analyses

For the descriptive analyses, the majority of disease notification rates were calculated using the 2006 census population of mainland New Zealand as the denominator. Where age-specific rates and age adjusted rates (by sex, ethnicity and Urban/Rural Profile) were calculated, the total New Zealand 2006 census population was used as the denominator. This was necessary because the required age-specific data were not available just for mainland meshblocks. The effect of using a different denominator population for selected parts of the analyses was negligible as the difference between mainland meshblock and total New Zealand populations was very small (4,014,801 and 4,027,698 respectively).

For analyses by deprivation and Urban/Rural Profile, those VTEC and cryptosporidiosis cases that could not be accurately assigned to a meshblock have been managed as missing data in the results. More details on the accuracy of meshblock assignment are provided later in the 'Ecological analyses' section.

Incident rate ratios (IRRs) were calculated in relation to the specified reference category (see results). Reference categories were chosen on the basis of being comparable with similar descriptive studides conducted in New Zealand (80, 83). Where crude rates only were used, these IRRs were calculated by direct division of the crude rates, relative to the reference category. Where age adjusted rates were calculated (by sex ethnicity and Urban/Rural Profile), IRRs were calculated using Poisson regression and adjusted for age. Poisson regression was used for age adjustment (rather than direct age standardisation) in order to calculate confidence intervals around the age-adjusted IRRs.

Confidence intervals around the IRRs of crude disease rates (by year, season, age, and deprivation) were calculated using exact methods. Confidence intervals around age-adjusted rates (sex, ethnicity and Urban/Rural Profile) were calculated using Poisson regression, with robust standard errors being used for Urban/Rural Profile analyses (see next section for rationale for the use of robust standard errors). Person-years over the 6-year period were used as the offset.

3.5.2 Ecological analyses

The analyses examining the association between animal density and VTEC and cryptosporidiosis occurred in two stages; these differed in regard to how the animal densities were categorised.

In the first stage, meshblocks were categorised into four animal exposure levels based on the density of each of the three animal types (dairy cattle, beef cattle and sheep). The exposure categories were: zero (no animals, the reference category), low, medium and high. Further details are provided in the results. While this categorisation resulted in the loss of information, it allowed for the possibility of non-linearity in the association between animal density and VTEC or cryptosporidiosis.

In order to link the case data with the animal density data, it was necessary for the case data to be assigned to a meshblock. However as mentioned, not all cases were accurately assigned. ESR has four categories of 'accuracy': 1) "exact", 2) "nearest street number", 3) "nearest street", or 4) (nearest) "Territorial authority" (TA). Cases with a meshblock assignment accuracy of 2) or 3), were unlikely to be misclassified in respect to animal exposure category as these cases are likely to be from urban areas (or areas with no animals). These cases were included in the current analyses. In contrast, cases with meshblock assignment accuracy of TA were likely to be misclassified in respect to animal exposure category. These cases are more likely to be from rural areas where it is more difficult to map a residence to a meshblock than in urban areas. Misclassification could result because all cases of 'TA' accuracy are automatically assigned to a meshblock in the centre of the territorial authority (109). Hence, cases with meshblock assignment accuracy of 'TA' were excluded from the current analyses. Subsequently, case data, denominator data, and animal density data were linked via the use of the meshblock unique identifier.

Disease rates were then calculated using the number of cases in the meshblocks of each animal density category as the numerator and the total population in the each animal density category as the denominator. Poisson regression modelling was used for univariate and multivariate analyses to calculate incidence rate ratios for each disease, by animal category. Person-years over the 6 year period, by meshblock category, were used as the offset.

Robust standard errors were used to calculate the confidence intervals for all Poisson models that examined the association between animal density and disease. Their use was to make allowance for violations of the assumptions of Poisson distribution that was likely to occur in the current analyses. These included a) lack of independence in disease rates between meshblocks (disease clustering), and b) variation in disease rates between meshblocks that were greater (or less) than expected with a Poisson distribution (overdispersion, or

underdispersion). While robust errors were a crude method of making allowance for these factors, they were likely to yield more accurate standard errors than a regression model that relied on the assumptions of a Poisson distribution.

Correlation between explanatory variables included in the full Poisson regression models, was examined using Spearman's rank correlation coefficients, the non-parametric equivalent of Pearson's correlation coefficients.

For the multivariate Poisson regression analyses, three separate models were developed, with each of the three animals (dairy cattle, beef cattle or sheep) as the main predictor. Separate models were required because of the high degree of correlation between the animal variables. To control for the potential confounders of age, ethnicity deprivation, and animal type, other covariates included were the percentage of the total meshblock population less than five years old, the percentage of European ethnicity, the New Zealand Deprivation index, and the density category of animals other than the main predictor (e.g.. beef and sheep if dairy was the main predictor). Details on how variables were removed from the models are included in the results.

Tests for trends in the multivariate analyses were conducted using Poisson regression with robust standard errors.

In the second stage of the analyses examining the association between animal density and disease, new categories of animal density for each animal type were created. For each animal type, those meshblocks with none of the specific animal type were excluded, and the remaining meshblocks were allocated into one of six animal density categories. Further details of the allocation are provided in the results. Using these new categories, data were analysed using the same methods as those used for the four category animal density measures. The reasons for performing the analyses after removing those meshblocks with no animals (by animal type) were three fold. The first was to reduce the effect meshblocks with no animals were having on the test for trends. The second was to make the included meshblocks more comparable to each other. In other words, in each model, the included meshblocks all had animals of the same type, therefore, the included areas were more likely to be similar in respect to other factors that may influence the relationship between animal density and disease. The third was to more reliably examine for a dose-response relationship between animal density and disease.

4 Results

This section begins with a description of the meshblocks included in the study, and describes the exclusion of cases. Following this, the results of the descriptive analyses and the ecological analyses are presented. This section concludes with a summary of the results.

4.1 Meshblocks included

For this study 38,743 mainland meshblocks that had a population of one or more people were included (Table 2). At the 2006 census 99.7% of the New Zealand population resided in mainland meshblocks.

Table 2: *Number of meshblocks and people usually resident within meshblocks (both with percentages of total), and number of meshblocks with a population of one or more people, by meshblock category.*

Meshblock category[1]	No.	%	Population[2]	%	No. meshblocks with pop. of zero	No. meshblocks with pop. of one or more
Mainland	40,466	97.76	4,014,801	99.68	1,723	38,743
Oceanic	165	0.40	147	0.00	154	11
Island	214	0.52	10,038	0.25	91	123
Inland waters	68	0.16	36	0.00	61	7
Inlet	428	1.03	732	0.02	358	70
Other	20	0.05	1,284	0.03	3	17
not classified	31	0.07	660	0.02	11	20
Total	41,392	100.00	4,027,698	100.00	2,401	38,991

[1] Meshblock category is based on the 2006 meshblock pattern defined by Statistics New Zealand

[2] Because of random rounding to base 3, the total New Zealand population varies dependent on the manner in which it is broken down by category

As a result of including only mainland meshblocks, during the study period:

- No VTEC cases were excluded
- Thirteen cryptosporidiosis were excluded (eight of these were from 'Island' meshblocks and five were from 'Other' meshblocks)

4.2 Case exclusions

Cases from mainland meshblocks were excluded on the basis of: 1) usually residing in a meshblock that had a 2006 census population of zero or 2) having a documented history of overseas travel within the incubation period of VTEC or cryptosporidiosis.

It is plausible that cases may been identified as being from a meshblock with a 2006 population of zero because of moving to a meshblock after the 2006 census. Alternatively, a population recorded as zero by Statistics New Zealand may actually be a population of one or two because of random rounding to base three by Statistics New Zealand (110). It was necessary to exclude cases from meshblocks with a population of zero as a disease rate cannot be calculated with a denominator population of zero.

No VTEC cases and only six cryptosporidiosis cases were excluded on the basis of being from a meshblock with a 2006 population of zero.

Twenty VTEC cases and 246 cryptosporidiosis cases were excluded on the basis of having a documented history of overseas travel within the incubation period of disease (see Table 3).

Table 3: *Number of notified VTEC and cryptosporidiosis cases (and percentage of total) for 2004-2009 inclusive, by documented history of overseas travel within incubation period of illness*[a]

	VTEC		Cryptosporidiosis	
Overseas travel	Number of cases	(%)	Number of cases	(%)
Yes	20	3.2	246	5.2
No	459	72.3	2,958	62.1
Unknown	156	24.6	1,556	33.7
Total	635	100	4,760	100

[a] Incubation period defined as 3-8 days for VTEC and 1-12 days for cryptosporidiosis

After case exclusions, 615 VTEC cases and 4,514 cryptosporidiosis cases were eligible for analysis.

4.3 Descriptive analyses

4.3.1 Incidence over study period

The average annual VTEC incidence rate over the study period was 2.55 cases per 100,000 per year. The average annual cryptosporidiosis incidence rate over the study period was 18.74 cases per 100,000 per year.

4.3.2 Incidence over time

The annual crude notification rate of VTEC increased from 2.2 per 100,000 in 2004 to almost 3.5 cases per 100,000 in 2009. While the test for linear trend (using Poisson regression) over the entire six year study period indicated that this increase was statistically significant ($p<0.001$), examination of Figure 1 shows that the rate was relatively stable until 2007.

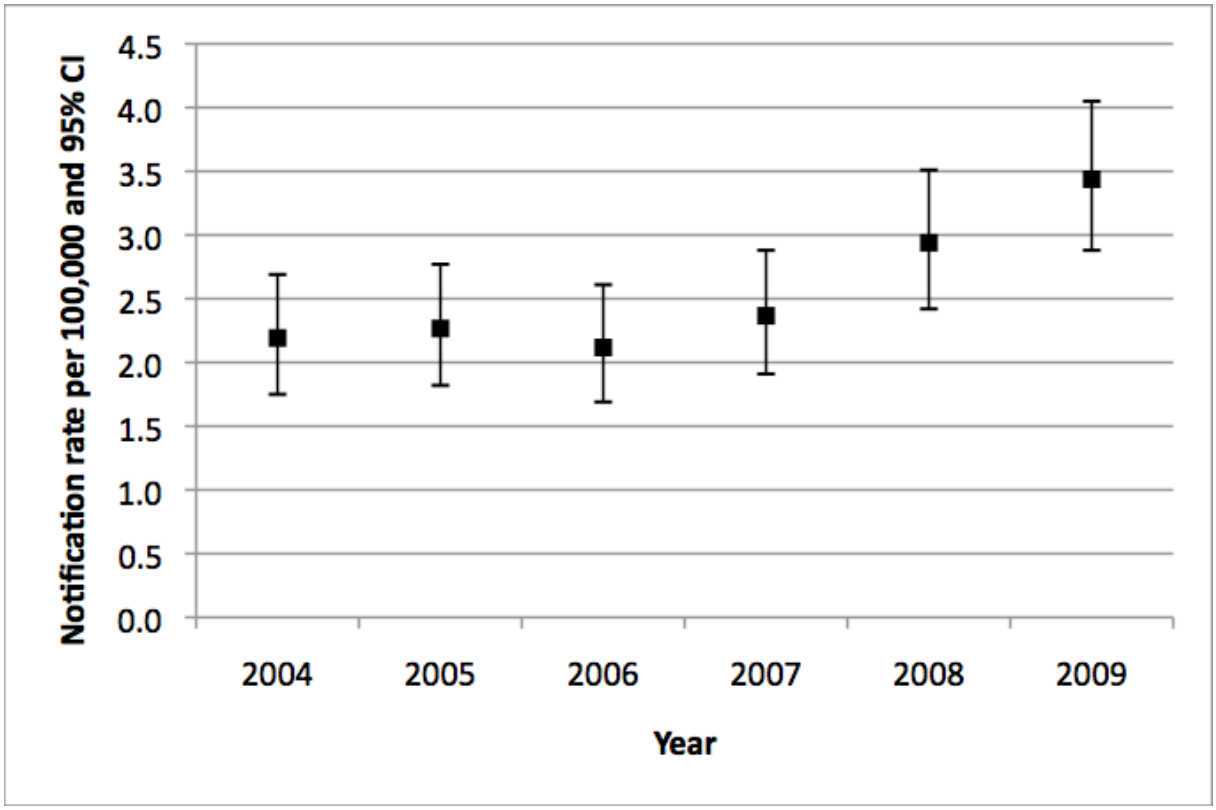

Figure 1: *Crude annual rates of VTEC notifications (cases per 100,000 population) and 95% confidence intervals, by year; 2004-2009*

The notification rate for cryptosporidiosis rose from 14.22 in 2004 to 20.25 per 100,000 in 2009, a statistically significant trend ($p<0.001$); however, this should be interpreted with caution because the cryptosporidiosis rates were above 20 cases per 100,000 for the years 2001-2003 and if included, these data were likely to have nullified this significant trend.

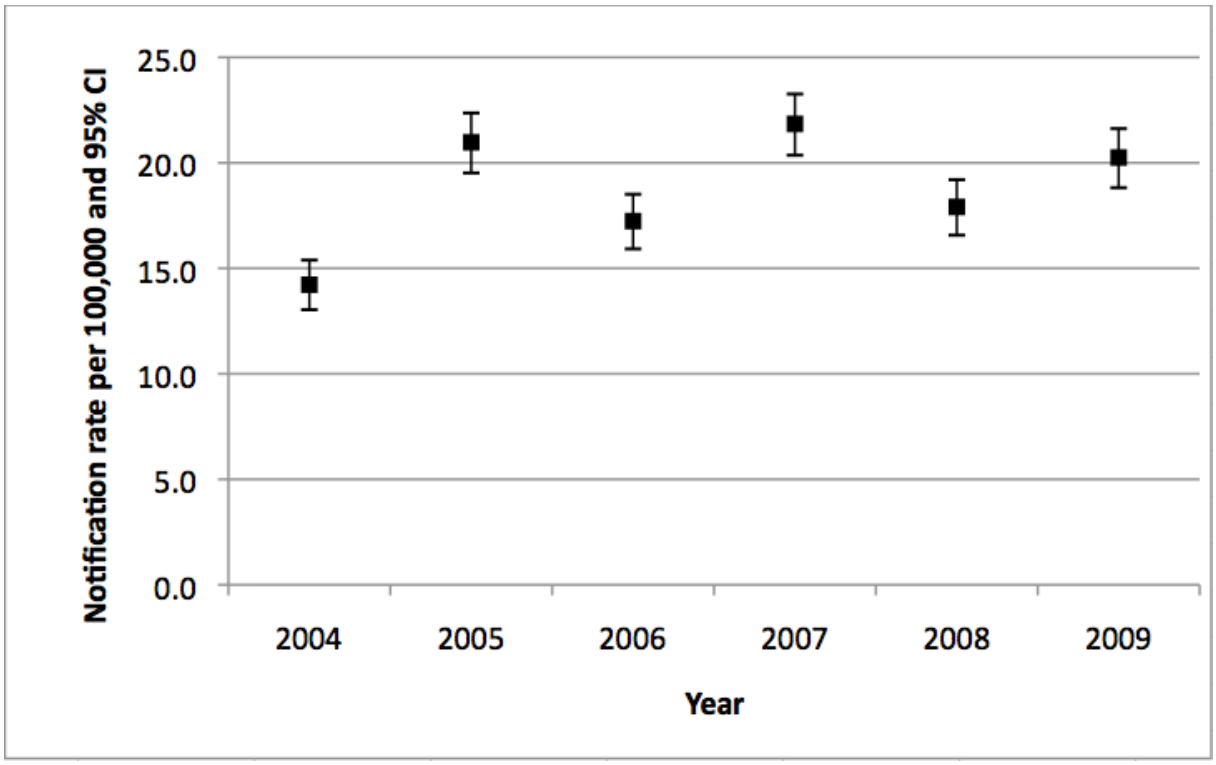

Figure 2: *Crude annual rates of cryptosporidiosis notifications (cases per 100,000 population) and 95% confidence intervals, by year; 2004-2009*

The crude notification rates of VTEC and cryptosporidiosis by season are shown in Table 4. With the rate in the summer months (December to February) as the reference, VTEC notifications were lower in winter and spring (rates approximately 50% and 20% lower, respectively). Autumn and summer rates were similar. For cryptosporidiosis, compared to summer, notifications rates were 4.6 times higher in spring, 35% higher in autumn and 24% higher in winter.

Table 4: *Number of VTEC and cryptosporidiosis cases, crude rate (average seasonal rate per 100,000), incidence rate ratios (IRR), and 95% confidence interval (95% CI), by season; years 2004-2009*

	VTEC					Cryptosporidiosis				
				95% CI					95% CI	
Season	No.	Rate	IRR	Lower	Upper	No.	Rate	IRR	Lower	Upper
Summer (Dec-Feb)	182	3.02	1.00			548	9.10	1.00		
Autumn (Mar-May)	195	3.24	1.07	0.87	1.32	741	12.30	1.35	1.21	1.51
Winter (June-Aug)	95	1.58	0.52	0.40	0.67	680	11.29	1.24	1.11	1.39
Spring (Sep-Nov)	143	2.37	0.79	0.63	0.98	2545	42.26	4.64	4.25	5.12

4.3.3 Notification rates by age, sex, ethnicity and deprivation

The notification rates of both VTEC and cryptosporidiosis were highest for children age 0-4 years (17.5 cases per 100,000 for VTEC and 107.2 cases per 100,000 for cryptosporidiosis), followed by children age 5-9 (3.49 per 100,000 for VTEC and 34.91 per 100,000 for cryptosporidiosis) (Table 5). The incidence rate ratio (IRR) of the youngest age group, compared with the reference age group of 20-29 was higher for VTEC (IRR=12.26) compared with cryptosporidiosis (IRR=7.06).

Table 5: *VTEC and cryptosporidiosis average annual notification numbers, crude rates (average annual rate per 100,000), incident rate ratios (IRR) and associated 95% confidence intervals (CI) by age group; years 2004-2009.*

	VTEC					Cryptosporidiosis				
Category	No.	Rate	IRR	95% CI		No.	Rate	IRR	95% CI	
				Lower	Upper				Lower	Upper
Age										
0-4	289	17.51	12.26	8.90	17.24	1,769	107.18	7.04	5.79	7.06
5-9	60	3.49	2.44	1.63	3.69	600	34.91	2.29	1.85	2.34
10-14	22	1.20	0.84	0.48	1.43	320	17.43	1.14	0.90	1.20
15-19	26	1.44	1.01	0.60	1.68	257	14.27	0.94	0.73	0.99
20-29	44	1.43	**1.00**			517	16.78	**1.00**		
30-39	33	0.95	0.67	0.41	1.07	528	15.22	1.00	0.80	1.03
40-49	37	1.02	0.71	0.45	1.13	249	6.84	0.45	0.35	0.47
50-59	32	1.10	0.77	0.47	1.24	150	5.14	0.34	0.25	0.37
60-69	38	1.93	1.35	0.85	2.13	75	3.81	0.25	0.18	0.29
70+	32	1.54	1.08	0.66	1.74	41	1.97	0.13	0.08	0.16

Missing/ unknown age group data: 2 VTEC cases, 8 cryptosporidiosis cases

Table 6 shows the age-standardised rates of both VTEC and cryptosporidiosis were slightly lower for males than females. For both diseases, age-standardised notification rates were highest for those of European ethnicity. For VTEC, age-standardised rates for Māori were approximately 65% lower than Europeans. For cryptosporidiosis, Māori rates were approximately 75% lower than Europeans.

Table 6: *VTEC and cryptosporidiosis average annual notification numbers, directly age standardised rates (average annual rate per 100,000), incident rate ratios (IRR) and associated 95% confidence intervals (CI), by sex and ethnicity; years 2004-2009*

Category	VTEC					Cryptosporidiosis				
	No.	Rate	IRR	95% CI lower	upper	No.	Rate	IRR	95% CI lower	upper
Sex										
Female	331	2.72	**1.00**			2,324	19.16	**1.00**		
Male	279	2.30	0.85	0.72	0.99	2,140	17.58	0.92	0.87	0.97
Ethnicity										
European	467	3.83	**1.00**			3,431	29.00	**1.00**		
Māori	64	1.65	0.35	0.27	0.46	362	7.86	0.26	0.23	0.29
Pacific	9	0.74	0.13	0.07	0.25	53	2.89	0.10	0.07	0.13
Asian	16	0.76	0.21	0.13	0.35	75	3.31	0.12	0.10	0.15
Other	4	0.16	0.04	0.02	0.11	19	0.82	0.03	0.02	0.04

Missing/ unknown sex data: 5 VTEC cases, 50 cryptosporidiosis cases

Missing/ unknown ethnicity data: 55 VTEC cases, 574 cryptosporidiosis cases

Crude rates of both diseases varied by deprivation score (Table 7). For VTEC, rates were similar for NZDep06 levels 1-6; and rates tended to be lower for NZDep06 levels 7-10. For cryptosporidiosis, rates progressively decreased with increasing deprivation. It was not possible to calculate age standardised rates for deprivation as complete age-specific population data was not available due to statistics New Zealand data confidentiality rules (110).

Table 7: *VTEC and cryptosporidiosis average annual notification numbers, crude rates (average annual rate per 100,000), incident rate ratios (IRR) and associated 95% confidence intervals (CI), by deprivation; years 2004-2009.*

Category	VTEC					Cryptosporidiosis				
	No.	Rate	IRR	95% CI Lower	Upper	No.	Rate	IRR	95% CI Lower	Upper
Deprivation scale										
1-2	115	2.32	1.00			1,082	21.86	1.00		
3-4	139	2.86	1.23	0.95	1.59	999	20.58	0.94	0.86	1.03
5-6	126	2.65	1.13	0.87	1.47	804	16.89	0.77	0.70	0.84
7-8	94	1.99	0.85	0.64	1.13	661	13.99	0.64	0.58	0.70
9-10	65	1.36	0.58	0.42	0.80	437	9.15	0.42	0.37	0.47

Missing/ Unknown deprivation data: 76 VTEC cases, 531 cryptosporidiosis cases (case data missing on basis of geographical matching not being exact)

4.3.4 Notification rates by rurality

Rurality was defined using the Urban/Rural Profile classification used by Statistics New Zealand for the 2006 census (103).

Table 8: *VTEC and cryptosporidiosis average annual notification numbers, directly age standardised rates (average annual rate per 100,000) and incidence rate ratios (with 95% CI) by Urban/Rural profile classification; years 2004-2009*

Urban rural profile	VTEC					Cryptosporidiosis				
	No	Rate	IRR	95% CI lower	upper	No.	Rate	RR	95% CI lower	upper
Main urban	299	1.71	**1.00**			2,166	12.32	**1.00**		
Satellite urban	18	2.31	1.32	0.80	2.16	133	17.29	1.39	1.12	1.72
Independent urban	68	2.65	1.53	1.04	2.25	570	23.31	1.89	1.51	2.36
Rural, high urban	42	5.75	3.38	2.30	4.96	204	28.25	2.28	1.90	2.75
Rural, mod. urban	37	4.09	2.39	1.29	4.40	327	35.99	2.98	2.49	3.56
Rural, low urban	80	5.91	3.53	1.79	6.99	577	45.03	3.58	2.62	4.88
Highly rural/remote	25	6.13	3.63	2.22	5.92	213	52.66	4.34	2.95	6.39
Urban total	385	1.84	**1.00**			2,869	13.75	**1.00**		
Rural total	184	5.44	2.97	1.85	4.77	1,321	40.07	2.88	2.24	3.70

Missing/ Unknown Urban/Rural profile data: 46 VTEC cases, 324 cryptosporidiosis cases (case data recorded as missing if accuracy of geographical case matching was at the level of "TA")

Table 8 shows that while the majority of the cases were notified from main urban areas, rates of both VTEC and cryptosporidiosis were higher in rural areas (IRR for VTEC 2.97 and for cryptosporidiosis 2.88). For VTEC, while there was some evidence of rates increasing as rurality increased, this trend was not consistent. By contrast. for cryptosporidiosis, rates increased as rurality increased, a statistically significant trend ($p<0.001$).

4.4 Description of animal data

Numbers of dairy cattle, beef cattle and sheep, by year (2004-2009) as collected by Agribase^{TM} for the study are shown in Table 9.

Table 9: *Numbers of dairy cattle, beef cattle and sheep recorded by Agribase™ for mainland meshblocks with a human population of one or more in New Zealand, by year; 2004-2009*

Year	Dairy	Beef	Sheep
2004	5,064,272	4,431,569	38,960,770
2005	5,248,924	4,454,569	38,717,190
2006	5,107,963	4,731,853	39,423,618
2007	5,888,507	4,860,819	39,349,652
2008	5,439,193	4,703,740	38,630,774
2009	5,128,094	4,599,476	37,857,273

The observed trend in the Agribase™ data was different from that data obtained from Statistics New Zealand (67) (see Table 10). In particular, Agribase™ recorded dairy cattle numbers *decreasing* from 2007 to 2009, whereas Statistics New Zealand recorded dairy cattle numbers *increasing.*

Table 10: *Numbers of dairy cattle, beef cattle and sheep cattle (in thousands) as recorded by Statistics New Zealand and Agribase™ by year; 2004-2009*

	Dairy cattle (1,000s)		Beef cattle (1,000s)		Sheep (1,000s)	
year	Stats NZ	Agribase	Stats NZ	Agribase	Stats NZ	Agribase
2004	5,153	5,064	4,447	4,432	39,271	38,961
2005	5,087	5,249	4,424	4,455	39,880	38,717
2006	5,170	5,108	4,439	4,732	40,082	39,424
2007	5,261	5,889	4,394	4,861	38,461	39,350
2008	5,578	5,439	4,137	4,704	34,088	38,631
2009	5,823	5,128	4,094	4,599	32,357	37,857

For years 2004-2006 and 2008-2009, Statistics New Zealand data was based on sample surveys of a total of 30,000 GST registered enterprises (includes livestock, horticulture, forestry and cropping). GST registration was compulsory above $60,000, so there was likely to be only partial coverage below this figure. For 2007, Statistics New Zealand data was based on an agricultural census rather than a survey

There are two main reasons that may explain the discrepancies in the data between Agribase™ and Statistics New Zealand (105).

1. Data collection change for Agribase™. After July the 1st 2005, Tuberculosis (Tb) testing that was contracted by the Animal Health Board was not performed solely by AsureQuality staff**.** Thus, in areas where Tb testing was not performed by

AsureQuality staff, dairy and cattle data was likely to be progressively less up-to-date unless the area had other projects in place for collecting farm data (such a rescue helicopter mailouts).

2. As mentioned in the methods, data was not collected every year for every farm included in the Agribase™ database. Thus, changes in the numbers of animals will not have been detected every year. Because of the data collection changes mentioned in above, this problem was likely to be particularly prominent for dairy and beef cattle. In this context, it is possible that data for recent large dairy conversions may not have been included in the Agribase™ database.

The Agribase™ data in Table 10 only includes mainland meshblocks with a human population of greater than zero yet the Statistics New Zealand data included all of New Zealand. However, the trends observed in the Agribase™ data were the same when data from all of New Zealand was analysed.

The mean animal numbers calculated across the years 2004-2009 were similar between the two databases. The Statistics New Zealand total numbers were lower than Agribase™ for beef and sheep. That was likely because Statistics New Zealand only collected data from G.S.T. registered farms, whereas Agribase™ does not have this exclusion. The slightly larger numbers of dairy cattle for Statistics New Zealand may be related to the factors previously discussed. After consideration of these issues, the mean number of animals by meshblock for 2004-2009 from the Agribase™ dataset was used. This corresponded to 5.31 million dairy cattle, 4.63 million beef cattle and 38.82 million sheep. It was not possible to use Statistics New Zealand data as these were not available at the geographical resolution of meshblock. Even for 2007 in which an animal census was carried out, Statistics New Zealand data was only available at the resolution of census area unit (111).

4.5 Ecological analyses

As mentioned in the Methods section, the analyses of the association between animal density (dairy cattle, beef cattle and sheep) and disease (VTEC and cryptosporidiosis) occurred in two stages. These differed according to the way animal density was categorised. The results of these analyses are presented below.

4.5.1 Using zero, low, medium and high animal density categories as explanatory variables

Each meshblock in the study was assigned a dairy cattle density, beef cattle density and sheep density category. These took the values of zero, low, medium or high (see Table 11). Except for the categories with zero density, the categories were determined on the basis of having equal numbers of meshblocks in each category. For all of these categories, the distribution of the animal density was highly positively skewed, thus the median density (as well as minimum and maximum values) were reported for each animal category (see Table 11). Table 11 also shows that in terms of numbers rather than rates, most cases of VTEC and cryptosporidiosis were notified from areas where there were no dairy cattle, beef cattle or sheep.

Table 11*: Median, minimum, and maximum animal density (animals per km^2), number of meshblocks, total human population and total number of accurately coded VTEC and cryptosporidiosis cases, by animal density category (dairy, beef and sheep)*

	Density (animals/km^2)			Number		Number of cases	
Category	Median	Min	Max	of meshblocks	Human pop.	VTEC	Crypto.
Dairy cattle							
Zero	0	0	0	32,785	3,568,839	407	2,966
Low	4	>0	<20	1,986	158,103	35	363
Med	54	20	<114	1,986	160,872	66	421
High	213	114	30,833	1,986	126,987	61	440
Beef cattle							
Zero	0	0	0	28,648	3,250,875	344	2,562
Low	6	0	<14	3,365	217,503	67	498
Med	25	14	<40	3,365	267,999	87	584
High	65	40	3,620	3,365	278,424	71	546
Sheep							
Zero	0	0	0	29,643	3,327,399	360	2,624
Low	6	0	<28	3,034	252,888	77	529
Med	79	28	<195	3,033	261,087	78	522
High	404	195	84,218	3,033	173,427	54	515

- Min and max densities are reported to integer values only. For each animal type, meshblock categories are mutually exclusive
- Zero refers to those meshblocks that have no dairy cattle, no beef cattle or no sheep, respectively
- 46 VTEC cases and 324 cryptosporidiosis cases excluded on basis of inaccurate geocoding (to level of TA only)

4.5.1.1 Univariate analyses

Univariate associations between each variable and meshblock VTEC and cryptosporidiosis rates were examined using Poisson regression with robust standard errors. The offset was person-years over the six year period (2004-2009) for each animal density category.

The univariate analyses (Table 12) showed that for all animals, rates of VTEC and cryptosporidiosis notifications were significantly higher in those areas with low, medium or high animal density, relative to those areas with an animal density of zero. The test for trend for all categories was also highly significant.

Table 12: *Crude VTEC and cryptosporidiosis incidence rate ratios (and 95% confidence intervals) and associated p-values, by categories of dairy density, beef density and sheep density. Years 2004-2009. P-values associated with the linear test for trend for each animal type are listed*

	VTEC					Cryptosporidiosis				
Explanatory variable	IRR	95% CI lower	upper	p value	Trend	IRR	95% CI lower	upper	p value	Trend
Dairy density										
Zero	**1.00**				<0.001	**1.00**				<0.001
Low	1.94	1.35	2.78	<0.001		2.76	2.44	3.12	<0.001	
Med	3.60	2.66	4.87	<0.001		3.15	2.79	3.55	<0.001	
High	4.21	3.13	5.67	<0.001		4.17	3.72	4.67	<0.001	
Beef density										
Zero	**1.00**				<0.001	**1.00**				<0.001
Low	2.91	2.16	3.92	<0.001		2.91	2.59	3.26	<0.001	
Med	3.07	2.35	4.01	<0.001		2.77	2.49	3.07	<0.001	
High	2.41	1.84	3.16	<0.001		2.49	2.25	2.75	<0.001	
Sheep density										
Zero	**1.00**				<0.001	**1.00**				<0.001
Low	2.81	2.16	3.66	<0.001		2.65	2.38	2.96	<0.001	
Med	2.76	2.08	3.66	<0.001		2.54	2.28	2.82	<0.001	
High	2.88	2.07	4.00	<0.001		3.77	3.39	4.19	<0.001	

46 VTEC cases and 324 cryptosporidiosis cases excluded on basis of inaccurate geocoding (to level of TA only)

4.5.1.2 Multivariate analyses

Variables were chosen for inclusion in the multivariate analyses based on the likelihood of them being confounders of the association between ruminant density and disease, as well as data availability at the meshblock level. Age, deprivation, ethnicity and animal density were the variables included. Age as a potential confounder was measured by the percentage of a meshblock population that were aged less than 5 years at the 2006 census (% <5years). Ethnicity was measured by the percentage of a meshblock population that were European ethnicity at the 2006 census (% European). Deprivation was measured by the NZDep06 index. Animal density (dairy beef and sheep) as potential confounders were measured based on zero, low, medium and high density categories.

Table 13 shows the Spearman's rank correlation coefficients of the variables included in the saturated multivariate model. Table 13 shows that coefficients were high between the zero, low, medium and high animal categories. In particular, beef cattle density was highly correlated with sheep density (Spearman's rank correlation = 0.88). The correlation coefficients between the animal categories and the potential confounders of % <5years, % European and NZDep06 were all less than 0.3, except between deprivation and ethnicity (0.58).

Table 13: *Spearman's rank correlation coefficient matrix of explanatory variables incorporated into full Poisson regression model using the zero, low, medium and high animal categories*

	Dairy cattle	Beef cattle	Sheep	% <5years	% European	NZDep06
Dairy cattle	1.00					
Beef cattle	0.72	1.00				
Sheep	0.69	0.88	1.00			
% <5years	0.04	0.01	0.01	1.00		
% European	0.22	0.24	0.28	-0.22	1.00	
NZDep06	-0.18	-0.21	-0.24	0.15	-0.58	1.00

- Animal variables (dairy cattle, beef cattle and sheep) correlation coefficients are based on none, low, medium and high categories of animal density
- % < 5years is the percentage of the total meshblock population that were age less than 5 years at the 2006 census
- % European is the percentage of the total meshblock population of European ethnicity at the 2006 census
- NZDep06 is the New Zealand Deprivation index of the meshblock (1-10, integer values)

Six regression models were developed. Dairy cattle, beef cattle and sheep were the main respective predictors for both VTEC and cryptosporidiosis as the outcome. All variables listed in Table 13 were included in the saturated Poisson regression model. Given the collinearity between the animal variables, only two out of the three animal variables were included in the final model. While keeping the main animal predictor in the model, variables were removed if their removal led to an improvement in model fit based on a reduction in the Akaike's information criteria (AIC), and if the variable was not acting as a confounder in the relationship between animal density and disease rates. A confounder was defined as a variable that when included changed the IRR of the highest density category relative to lowest by 10% or more. Removal of variables was in a step-wise fashion. The variable first removed was that which, when removed, led to the greatest improvement in model fit. The removal of additional variables was based on the same methods.

Using these methods for both diseases, with dairy as the main predictor, beef was retained in the model. For beef as the main predictor, dairy was retained; and for sheep, dairy was retained. For all final models with VTEC as the outcome, NZ Dep06 was removed because its inclusion did not improve model fit, nor was it acting as a confounder in the association between the animal density categories and VTEC rates. For all final models for cryptosporidiosis, NZDep06 was retained as its inclusion improved model fit. 'Percentage less than 5 years' and 'percentage European' were retained in all models, as they acted both as (positive) confounders in the relationship between ruminant density and disease and their inclusion improved model fit.

As it was possible that the relationship between 'percentage less than five years' and disease risk was non-linear because of person to person disease transmission, 'percentage less than 5 years' was introduced into the full multivariate models as both a linear and quadratic term. As the quadratic term was not statistically significant, it was assumed that the relationship between 'percentage less than 5 years' and VTEC disease risk was linear, and the quadratic term was not included in subsequent models.

The results for the multivariate analyses using the low, medium and high categories for both diseases are listed in Table 14. The adjusted IRRs are close to half that of the unadjusted IRRs, indicating positive confounding by the other covariates in the multivariate models.

Table 14: *Adjusted VTEC and cryptosporidiosis incidence rate ratios (and 95% confidence intervals) and associated p-values, by categories (zero, low, medium and high) of dairy density, beef density and sheep density. Years 2004-2009. P-values associated with the linear test for trend for each animal type are listed*

	VTEC					Cryptosporidiosis				
Explanatory variable	Adj. IRR[(1)]	95% CI lower	upper	P-value	Trend	Adj. IRR[(2)]	95% CI lower	upper	P-value	Trend
Dairy density										
Zero	**1.00**				<0.001	**1.00**				<0.001
Low	1.12	0.70	1.77	0.640		1.44	1.24	1.69	<0.001	
Med	2.09	1.38	3.15	0.000		1.69	1.45	1.96	<0.001	
High	2.34	1.57	3.48	0.000		2.36	2.05	2.72	<0.001	
Beef density										
Zero	**1.00**				0.107	**1.00**				<0.001
Low	1.60	1.09	2.36	0.017		1.46	1.26	1.69	<0.001	
Med	1.74	1.24	2.46	0.002		1.43	1.25	1.64	<0.001	
High	1.39	0.93	2.07	0.105		1.35	1.17	1.56	<0.001	
Sheep density										
Zero	**1.00**				0.019	**1.00**				<0.001
Low	1.55	1.06	2.26	0.023		1.38	1.21	1.59	<0.001	
Med	1.58	1.10	2.28	0.014		1.45	1.26	1.66	<0.001	
High	1.60	1.03	2.47	0.035		1.99	1.73	2.30	<0.001	

(1) Adjusted for age, ethnicity, and animal types. For VTEC, deprivation did not act as a confounder nor improve model fit so was not included in the models

(2) Adjusted for age, ethnicity, animal type and deprivation

46 VTEC cases and 324 cryptosporidiosis cases were excluded on the basis of inaccurate geocoding (to level of TA only)

For dairy cattle, VTEC notifications were not significantly more common in the areas with low density compared to none, but were in the areas of medium or high density. Overall, there was a statistically significant increase in the IRRs with increasing dairy density ($p<0.001$). Cryptosporidiosis notifications were significantly more common in the areas with low density compared to none, and again there was a statistically significant trend in the IRRs, with increasing dairy density ($p<0.001$).

For beef cattle, VTEC notifications were significantly more common in the areas with low and medium density compared to none, but not in the areas of high density. Overall, there was no statistically significant increase in the IRR with increasing beef cattle density.

Cryptosporidiosis notifications were significantly more common in all areas with beef cattle compared to those with none, but the IRRs did not increase in a consistent manner as density increased. While the test for trend was significant ($p<0.001$), it is likely that this result is overly influenced by step up increase in disease rates from the zero to low beef category.

For sheep, VTEC notifications were significantly more common in those areas with low, medium or high density compared to none. While there was a statistically significant trend in the increase in the IRRs with increasing sheep density ($p=0.019$), examination of the table suggests that this was driven by the step up in risk from zero to low density areas, rather than a dose response with increasing density. In contrast, cryptosporidiosis notifications were significantly more common in all the categorised areas with any sheep density compared to none, and there was a statistically significant trend in the IRRs with increasing sheep density ($P<0.001$).

4.5.2 Using six category animal density categories as explanatory variables

As outlined in the Methods, meshblocks with no dairy cattle, beef cattle or sheep were removed from the respective animal analyses, and the remaining meshblocks were re-categorised (see Table 15). Having six categories, rather than three, allowed better examination of the trends. Categories were determined on the basis of relatively similar numbers of cases in each (allowing more for the reference category), while creating animal density ranges that were easily understandable. Because there were more meshblocks with lower animal densities than higher densities, the range of densities included for the lower density categories are smaller than the higher density categories.

Table 15: *Median density (animals per km^2), number of meshblocks, total human population and number of accurately coded VTEC and cryptosporidiosis cases by ruminant density categories (dairy cattle, beef cattle and sheep).*

Density range (animals/km^2)	Median density (animals/km^2)	Number of meshblocks	Human pop.	Number of cases	
				VTEC	Crypto.
Dairy cattle					
>0-<25	4.6	2,156	174,471	41	420
25-<50	37.3	738	57,729	30	130
50-<100	73.8	891	72,627	26	199
100-<200	142.0	1,060	76,557	32	250
200-<300	243.5	702	44,181	19	168
300+	349.3	411	20,397	14	57
Beef cattle					
>0-<12.5	5.2	3,134	200,712	64	457
12.5-<25	18.0	1,960	154,395	52	339
25-<37.5	30.9	1,433	114,270	36	246
37.5-<50	43.4	1,005	88,635	17	202
50-<75	60.3	1,267	108,888	30	217
75+	102.0	1,296	97,026	26	167
Sheep					
>0-<25	5.8	2,898	240,990	72	502
25-<50	35.1	911	83,496	24	148
50-<100	71.5	1,140	106,518	28	202
100-<200	142.7	1,174	87,495	34	212
200-<400	287.3	1,431	92,970	28	256
400+	587.6	1,545	75,837	23	246

VTEC cases excluded on basis of inaccurate geocoding: 30, 46 and 43 from dairying, beef and sheep areas respectively. Cryptosporidiosis cases excluded on basis of inaccurate geocoding: 175, 280 and 259 from dairying, beef and sheep areas respectively. Note that these cases are not mutually exclusive.

4.5.2.1 Univariate analyses

The results of the six separate univariate analyses of the association between ruminant density (using 6 categories of ruminant density) and VTEC and cryptosporidiosis are presented in Table 16 for reference.

Table 16: *Crude VTEC and cryptosporidiosis incidence rate ratios (and 95% confidence intervals calculated using robust standard errors) and associated p-values, by categories of dairy density, beef density and sheep density. Years 2004-2009. P-values associated with the linear test for trend are listed*

	VTEC					Cryptosporidiosis				
Density category (animals/km^2)	IRR	95% CI lower	upper	p-value	Trend	IRR	95% CI lower	upper	p-value	Trend
Dairy cattle										
>0-<25	**1.00**				0.003	**1.00**				<0.001
25-<50	2.21	1.30	3.75	0.003		1.02	0.86	1.21	0.800	
50-<100	1.52	0.87	2.66	0.139		1.23	1.02	1.47	0.026	
100-<200	1.78	1.09	2.92	0.022		1.34	1.17	1.55	<0.001	
200-<300	1.83	1.02	3.27	0.042		1.44	1.23	1.70	<0.001	
300+	2.92	1.46	5.83	0.002		1.57	1.25	1.96	<0.001	
Beef cattle										
>0-<12.5	**1.00**				0.198	**1.00**				<0.001
12.5-<25	1.06	0.69	1.61	0.799		0.87	0.75	1.01	0.073	
25-<37.5	0.99	0.62	1.57	0.960		0.80	0.69	0.92	0.001	
37.5-<50	0.60	0.35	1.04	0.070		0.72	0.62	0.84	<0.001	
50-<75	0.86	0.53	1.41	0.556		0.73	0.64	0.84	<0.001	
75+	0.84	0.52	1.36	0.481		0.70	0.60	0.82	<0.001	
Sheep										
>0-<25	**1.00**				0.711	**1.00**				<0.001
25-<50	0.96	0.58	1.59	0.881		0.79	0.67	0.95	0.010	
50-<100	0.88	0.52	1.50	0.636		0.74	0.64	0.86	<0.001	
100-<200	1.30	0.84	2.01	0.234		1.01	0.88	1.16	0.882	
200-<400	1.01	0.61	1.67	0.975		1.25	1.08	1.44	0.003	
400+	1.02	0.60	1.73	0.956		1.55	1.36	1.75	<0.001	

4.5.2.2 Multivariate analyses

The Spearman's rank correlation coefficients of the variables included in the multivariate model (Table 17) showed that the correlation coefficients between animal categories using six categories were a lot lower than the coefficients obtained when the zero, low, medium, high categories were used (see Table 13).

Table 17: *Spearman's rank correlation coefficient matrix of explanatory variables incorporated into full Poisson regression model using six category animal densities*

	Dairy cattle	Beef cattle	Sheep	% <5years	% European	NZDep06
Dairy cattle	1.00					
Beef cattle	-0.20	1.00				
Sheep	-0.38	0.19	1.00			
% <5years	0.11	-0.07	-0.01	1.00		
% European	-0.02	-0.09	0.22	-0.07	1.00	
NZDep06	-0.02	-0.05	-0.21	0.05	-0.46	1.00

- Animal variables (Dairy cattle, beef cattle and sheep) coefficients are based on six category animal density (those meshblocks with zero animals have been excluded)
- % < 5years is the percentage of the total meshblock population that were age less than 5 years at the 2006 census
- % European is the percentage of the total meshblock population of European ethnicity at the 2006 census
- NZDep 06 is the New Zealand Deprivation index of the meshblock (1-10, integer values)

For the multivariate analyses, all variables listed in Table 17 were included in the saturated Poisson regression model. The model building proceeded in a similar manner as the previous multivariate analyses. The main difference was that collinearity was not such as issue; thus the animal variables were treated in the same way as all other variables included in the model. To reiterate, a saturated model was developed six separate times, with dairy cattle, beef cattle and sheep being the main respective predictors for both VTEC and cryptosporidiosis. Variables were then removed if their removal led to an improvement in model fit based on a reduction in the AIC, and they were not acting as a confounder in the relationship between animal density and disease rates. Removal of variables was in a step-wise fashion. The variable first removed was that which, when removed, led to the greatest improvement in model fit. The removal of additional variables was based on the same methods.

Using these methods for VTEC, NZDep06 was removed first from the models with dairy and sheep as the explanatory variables, as it neither contributed to model fit nor was acting as a confounder. With dairy as the explanatory variable, beef was subsequently removed, and for sheep, beef was subsequently removed. For both dairy and sheep, 'percentage less than 5 years' and 'percentage European' contributed to model fit so were retained. With beef as the explanatory variable, all variables in the saturated model contributed to model fit so all were retained in the final model. For cryptosporidiosis, all variables in the saturated models

contributed to model fit so all variables were included in the final models. For both diseases, 'percentage less than 5 years' as a quadratic term was introduced into the saturated regression model but was not significant and therefore, was not included in subsequent models

The results of the multivariate analyses are presented in Table 18. The adjusted IRR of VTEC and cryptosporidiosis were relatively similar to the unadjusted IRR observed in Table 16. This indicated there was minimal confounding by the variables included in the models, or confounding in opposite directions that cancelled each other out. This similarity between adjusted and unadjusted IRRs in these analyses (with six categories of animal density) is in contrast with the previous analyses (with zero, low, medium, high animal density categories) where the adjusted IRRs were close to half that of the crude IRRs.

The multivariate analyses showed that within dairy cattle areas, VTEC notification rates were significantly higher in the areas with dairy density of 25-<50 dairy cattle/km^2 and 300+ dairy cattle/km^2, relative to the reference category of >0-<25 dairy cattle/km^2. Rates in the areas with between 50-<300 dairy cattle/km^2 did not have significantly higher rates than the reference category. While the test for trend was significant (p=0.019), the relationship between dairy density and VTEC did not appear to be consistent with a dose response relationship. In comparison, for cryptosporidiosis, there was a steady increase in IRRs with increasing dairy density level that was highly statistically significant (p<0.001).

Within beef areas, for VTEC there was no statistically significant difference in the IRRs for any of the categories of density. While there was no significant trend (p=0.075), there was a tendency for a IRRs to decrease as beef density increased. For cryptosporidiosis, there was a statistically significant test for trend and this trend was negative (p<0.001). Examination of the table indicates that cryptosporidiosis rates decreased in a relatively regular manner as beef density increased.

Within sheep areas, for VTEC, there was no evidence of a clear relationship between sheep density and VTEC notification rates. However, for cryptosporidiosis, notification rates increased once sheep density was greater than 100 sheep/km^2. While there was a decrease in cryptosporidiosis rates for the categories 25-<50 and 50-<100 sheep/km^2, relative to the reference category, the decrease was statistically non-significant. Overall, for the relationship between sheep density and cryptosporidiosis, the test for trend was significant (p<0.001), and

there was a linear relationship between sheep density and cryptosporidiosis risk, at least once sheep density reached 100sheep/km^2.

Table 18: *Adjusted VTEC and cryptosporidiosis incidence rate ratios (and 95% confidence intervals) and associated p-values, by categories of dairy density, beef density and sheep density. Years 2004-2009. P-values associated with the linear test for trend for each animal type are listed*

	VTEC					Cryptosporidiosis				
Density Category (animals/km^2)	Adj. IRR[(1)]	95% CI lower	upper	p value	Trend	Adj. IRR[(2)]	95% CI lower	upper	p value	Trend
Dairy cattle										
>0-<25	**1.00**				0.019	**1.00**				<0.001
25-<50	2.18	1.25	3.81	0.006		1.03	0.89	1.19	0.682	
50-<100	1.36	0.73	2.51	0.333		1.18	1.00	1.39	0.050	
100-<200	1.63	0.94	2.83	0.081		1.33	1.15	1.53	<0.001	
200-<300	1.76	0.92	3.37	0.086		1.42	1.18	1.70	<0.001	
300+	3.33	1.39	7.98	0.007		1.48	1.17	1.86	0.001	
Beef cattle										
>0-<12.5	**1.00**				0.075	**1.00**				<0.001
12.5-<25	1.13	0.64	1.99	0.325		0.95	0.82	1.12	0.561	
25-<37.5	0.99	0.52	1.90	0.329		0.81	0.69	0.95	0.009	
37.5-<50	0.67	0.32	1.40	0.252		0.77	0.66	0.91	0.002	
50-<75	0.64	0.32	1.29	0.227		0.77	0.65	0.91	0.002	
75+	0.68	0.33	1.42	0.256		0.75	0.62	0.90	0.002	
Sheep										
>0-<25	**1.00**					**1.00**				<0.001
25-<50	1.31	0.73	2.34	0.361	0.120	0.87	0.71	1.06	0.164	
50-<100	1.31	0.72	2.41	0.379		0.87	0.73	1.05	0.144	
100-<200	1.30	0.75	2.27	0.352		1.31	1.12	1.54	0.001	
200-<400	1.26	0.71	2.23	0.435		1.40	1.19	1.65	<0.001	
400+	0.99	0.44	2.22	0.983		1.59	1.36	1.85	<0.001	

(1) Adjusted for age, ethnicity, and animal types and (for beef only). For dairy and sheep, deprivation did not act as a confounder nor improve model fit so was not included in the VTEC models

(2) Adjusted for age, ethnicity, animal type and deprivation

4.6 Summary of results

4.6.1 Descriptive analyses

VTEC and cryptosporidiosis notification rates increased over the study period (2004-2009). Relative to the reference categories, rates for both diseases were highest amongst young children, particularly those less than 5 years old; those of European ethnicity; those in the least deprived areas, and those from rural areas. In addition, rates of both diseases varied by season although the patterns were different. For VTEC, notification rates were higher in summer and autumn relative to winter and spring, while for cryptosporidiosis, rates were higher in spring relative to summer, winter and autumn.

4.6.2 Ecological analyses

This study found that rates of VTEC and cryptosporidiosis tended to be significantly higher in those areas that have any of the three animal types (sheep, beef cattle or dairy cattle) relative to those that have no animals of the respective types. For some of the analyses with no animals (by animal type) as the reference category, there was a step up in disease risk as the animal density increased from zero to low. These included the relationship between beef density and both VTEC and cryptosporidiosis, as well as the relationship between sheep and VTEC. In contrast, for other analyses with zero animals as the reference category, there was no comparable step up in disease risk. These included the relationship between dairy density and both VTEC and cryptosporidiosis, as well as the relationship between sheep density and cryptosporidiosis.

Within dairying areas, relative to the lowest dairy density areas (between 0 and 25 animals/km^2 exclusive), adjusted VTEC rates were significantly higher in areas where density was between 25 and 50 animals/km^2 (IRR.2.21; 95% CI 1.30-3.75) and greater than 300 animals/km^2 (IRR 3.33; 95% CI 1.39-7.98). However, there was no clear dose response. In contrast, adjusted cryptosporidiosis rates increased incrementally as dairy density increased (p for trend < 0.001).

Within beef cattle areas, adjusted VTEC rates did not vary significantly by changes in beef density. Conversely, adjusted cryptosporidiosis rates *decreased* in a relatively steady manner as beef density increased and the trend was highly significant (p<0.001).

Within sheep areas, there was no evidence that adjusted VTEC rates varied by changes in sheep density. In contrast, adjusted cryptosporidiosis rates *increased* significantly once sheep density was greater than 100sheep/km^2.

5 Discussion

As well as conducting a descriptive analysis of notified VTEC and cryptosporidiosis in New Zealand, this study performed ecological analyses of the relationship between the density of the major farm animals in New Zealand (dairy cattle, beef cattle and sheep), and the rates of notified VTEC and cryptosporidiosis.

This chapter first highlights the strengths and weaknesses common to both the descriptive and ecological analyses. With this background, the findings of the descriptive analyses are then discussed in relation to other published studies. Subsequently, the specific strengths and weaknesses of the ecological analyses are outlined. Following a discussion of the ecological analyses, the implications of the findings of this study are considered, and future research needs highlighted.

5.1 Strengths and weaknesses common to descriptive and ecological analyses

Both the descriptive and ecological analyses included all of mainland New Zealand. When compared with a more geographically limited study, there is likely to be greater certainty that the results are not due to a region-specific aberration, as could occur if there were abnormally high notification rates of VTEC or cryptosporidiosis through more testing by a particular health service.

Notification data from a six-year period (2004-2009) for both VTEC and cryptosporidiosis were used in the analyses. There were relatively large numbers of cases included, with consequent reduction of random error in statistical estimates. These data also allowed assessment of trends over time for the descriptive analyses. Limitations in the animal data (discussed later) meant that trends over time were not able to be assessed for the ecological analyses.

Cases with a recent history of overseas travel were excluded from analyses. While these exclusions may not have been complete, relevant exposures were most likely to have occurred within New Zealand.

Analyses included both sporadic and outbreak cases of VTEC and cryptosporidiosis, as it was not possible to reliably distinguish between them. Therefore, this study was not able to assess whether the risk of VTEC or cryptosporidiosis in New Zealand varied by sporadic or outbreak case status.

The numbers of notified cases were almost certainly less than the actual number of cases. An Australian study found that for VTEC, under-notification occurs by a factor of about eight (112), and the findings of UK study based on campylobacter suggest that under-notification rates for cryptosporidiosis are likely to be similar (113). Within New Zealand, the Acute Gastrointestinal Illness (AGI) Study undertaken for the New Zealand Food Safety Authority showed that the undernotification of AGI, occurs by a factor of 250 (114). While this study could not conclude on the undernotification rates of specific pathogens such as VTEC or cryptosporidiosis, it showed that the undernotification of AGI in NZ is comparable to that of UK and Australia. This suggests the findings of the Australian and UK studies on VTEC and cryptosporidiosis notifications cited above are likely to be generalisable to New Zealand. For the current analyses, under-notification may not only have been problematic due to reduction in case numbers and therefore study power, but also if under-notification was biased. The effect this possible bias may have had on the analyses will be discussed later.

5.2 Discussion of results of descriptive analyses

The findings of the descriptive analyses are comparable to those reported in New Zealand by Baker et al. for both VTEC (20, 79) and cryptosporidiosis (80, 83). This is unsurprising as similar sources of data were used, and those cases excluded from the current analyses (overseas cases and cases from non-mainland meshblocks) and not from the previous New Zealand analyses, were a small proportion of the total number of cases.

In this present study, rates of VTEC and cryptosporidiosis for all age groups were not significantly different between males and females, consistent with New Zealand and international observations (13-15, 79, 83). The relatively high rates amongst children are also consistent with New Zealand and international observations for both VTEC (13, 15, 79) and cryptosporidiosis (14, 83, 115).

This study also showed that those of European ethnicity had at least 2.5 times the notification rates of VTEC and cryptosporidiosis compared with Māori and other ethnicities. However, the higher rates of disease observed amongst Europeans relative to other ethnicities may be due, in part, to bias in notification rates. This is supported by New Zealand research showing similar rates of gastrointestinal illness between Māori and other ethnicities. For example, in an analyses of intestinal infection hospitalisations, non-Māori (mostly European) had rates only 20% higher than Māori (116) and in the AGI study cited above, non-Māori had slightly lower community diagnosied AGI rates compared to Māori (114). This likely under-notification of disease in non-European ethnic groups relative to Europeans may occur because of poorer access to primary care services. For Māori, the idea of poorer access is supported by findings of the National Primary Medical Care Survey which showed Māori had fewer visits to a GP and were less likely to have specific diagnostic tests ordered when compared with non-Māori (117). As those in more deprived areas are more likely to have barriers to accessing to health care than those in less deprived areas (118), this may also explain why rates of VTEC and cryptosporidiosis are lower amongst those living in more deprived areas.

There was a significant increase in both VTEC and cryptosporidiosis rates over the study period (2004-2009). While possible reasons for this include expansion of the dairy cattle industry over the study period (67), it is important to consider that for cryptosporidiosis, rates were higher in 2001-2003 (80). This suggests agricultural expansion may not have been a key driver of the increase in cryptosporidiosis observed during the study period. However, it is also possible that the higher cryptosporidiosis rates in 2001-3 were due to predominance of *C. hominis*, which is unlikely to be associated with cattle, and more likely to be associated with both urban areas (70) and swimming pool-acquired infections (70, 82). Indeed, outbreaks of *C. hominis* occurred during 2001 and 2002 (70). Further data are required to assess whether or not the increases in VTEC and cryptosporidiosis over the study period may have been due, in part, to changes in the agricultural make-up of New Zealand.

Rates of notified VTEC and cryptosporidiosis were significantly higher in rural areas than urban areas. Internationally, a similar 'urban rural gradient' has been observed for both VTEC (85, 87) and cryptosporidiosis due to *C. parvum* (86). It is unlikely that this gradient was explained by differences in notification rates by rurality as barriers to primary care access (which is likely to influence notification rates) are greater, not less, in rural areas compared with urban (119). If anything, the urban-rural gradient was likely to be underestimated by

notification bias. The urban-rural gradient was also likely to have been further underestimated by bias in the accuracy of geocoding by rurality. To clarify, recall that a case could only be included in the urban-rural (or animal density) analyses in the current study if it was accurately geocoded. Based on New Zealand research of enteric disease notifications (120), cases from rural areas were up to four times more likely to be innaccurately geocoded than cases from urban areas. Hence, it follows that rural cases were up to four times more likely to be excluded from the current analyses than urban cases which would have had the effect of underestimating the size of the urban rural gradient for VTEC and cryptosporidiosis by up to a factor of four.

The seasonal peaks of VTEC and cryptosporidiosis were different. However, for both diseases, the highest notification rates corresponded with the timing of maximal excretion of VTEC and *Cryptosporidium* by common reservoir species. For VTEC, the highest rates were in summer and autumn. Excretion of VTEC is highest in young infected cattle typically less than one year but older than three months of age (11, 15). Therefore, after a typical spring birth, young cattle will begin to excrete VTEC maximally in summer. While young cattle have the highest excretion rates, the excretion of VTEC in the summer and autumn is elevated for all cattle. This elevated excretion is thought to be related to the increase of VTEC in cattle feed and water over these warmer months (11). For cryptosporidiosis, the highest rates of disease occurred in spring. This aligns with the season in which newly born calves excrete vast numbers of oocytes (up to 10^6 per gram of faeces) (39). Calves are thought to be responsible for the majority of *Cryptosporidium* shed on farms (43).

This study did not identify an autumn peak of cryptosporidiosis, which suggests an absence of significantly sized swimming pool outbreaks that have previously occurred in New Zealand (70).

The seasonal correlation between high human disease rates and maximal shedding by animals supports zoonotic transmission rather than person-to person transmission in maintaining the incidence of VTEC and cryptosporidiosis in New Zealand.

5.3 Strengths and weakness of ecological analyses

This study is the first to investigate the relationship between specific animal densities, and human VTEC or cryptosporidiosis rates in New Zealand.

An ecological study design was appropriate to answer the ecological question of whether populations living in areas of high cattle or sheep density were at increased risk of VTEC or cryptosporidiosis. However, if inferences are to be made from any ecological study at the individual level, the role of ecological fallacy or ecological bias needs consideration. In this context, care needs to be taken in assuming the measure of animal density can act as a surrogate for individual contact with VTEC or *Cryptosporidium* excreted by animals.

One strategy for minimising ecological bias is to use small geographical units (95); and for this study meshblocks, the smallest geopolitical unit available in New Zealand, were used However, while the median meshblock size was only 0.07km^2 some meshblocks were up to 2000km^2, so ecological bias could still be an issue when making inferences on individuals' risk of disease. Nevertheless, the geographical units in this study were smaller than in similar previous studies (for example (83, 86, 88)), so this study is likely to better reflect individual risk of animal exposure.

One downside in using small geographical units is that, in comparison to larger units, it is more likely that the location of a case from the disease register does not correspond to the location of the source of the infection. While this 'migration bias' may have resulted in some cases being assigned to meshblocks some distance from the original infection source, for diseases with a short incubation period such as VTEC or cryptosporidiosis, this form of bias is less likely to have a major impact than for diseases with a long incubation period or latency.

As highlighted in the Results section, changes in the collection of animal data over the study period, particularly after 2005 (105), meant it was possible some large dairy farms were under-reported. Consequently, several populations included in this study may have been incorrectly classified as being unexposed to dairy cattle. This would bias the relationship between dairy density and disease towards the null. The changes in the collection of animal data also meant it was not possible to accurately assess if changes in animal density over the study period were related to changes in the incidence rates of VTEC or cryptosporidiosis over time.

This study adjusted for several possible confounders, including age, deprivation, ethnicity, be associated with VTEC and cryptosporidiosis rates. Analyses also attempted to separate out the effects of different types of farm animals, so included density of dairy cattle, beef cattle and sheep in models. Multicollinearity in some models meant that separation of these effects may not have always been complete. Confounding control was also limited by missing population level data and by the standard ecological study problem of using group level data on confounders rather than individual level data (24).

This study did not adjust for the density of agricultural animals other than dairy cattle, beef cattle sheep. The literature indicates animals such as deer and goats may be potential reservoirs for VTEC (17) and *Cryptosporidium* (29, 39). However, there are relatively small numbers of deer and goats in New Zealand (67), and for cryptosporidiosis at least, they are unlikely to be major reservoir hosts (29, 94). Thus, relative to cattle or sheep, they are unlikely to contribute greatly to the overall reservoir pool of the VTEC or *Cryptosporidium.* Also, it is unlikely that the density of these animals will increase as dairy cattle or sheep increase.

Other potential confounders not adjusted for were wild animals, humans and treatment of drinking water. Wild animals, including possums, birds and rats carry *Cryptosporidium* in New Zealand (44), and overseas studies show VTEC is carried by possums (17), birds and rats (119). However, the presence of wild animals is unlikely to increase with increasing cattle or sheep density; in fact the opposite is more likely. As such, wild animals as well as agricultural animals such as deer and goats, are unlikely to explain the results of those analyses in which an increase in disease risk was associated with an increase in animal density (for example, the association between sheep or dairy density and cryptosporidiosis).

Humans can excrete VTEC and *Cryptosporidium* and can transmit the pathogens to each other via direct (63, 64) or indirect contact, including through water contaminated by human faeces (17, 25). Thus, it is feasible that VTEC or *Cryptosporidium* excreted by humans could confound the relationship between animal density and disease. However, because the seasonal peak of VTEC and cryptosporidiosis aligns with the maximal seasonal excretion by animal reservoirs it is likely that pathogens excreted by animals, rather than humans, may be the most important in causing disease. For cryptosporidiosis this seasonal peak was particularly prominent, and *C. parvum* (and not human derived *C. hominis*) is the dominant species in rural New Zealand (70). Therefore, for cryptosporidiosis it is unlikely that the relationship with animal density was confounded by pathogens shed by humans, particularly for those

analyses where there was a dose response between animal density and disease. On the other hand for VTEC, the situation is less clear, especially considering the seasonal peak was not as prominent.

Treatment of water is required to ensure that water contaminated by VTEC or cryptosporidiosis is safe to drink. The importance of drinking water treatment as a determinant of VTEC and cryptosporidiosis is illustrated by international studies showing that water treatment failure has been frequently associated with outbreaks of both VTEC (17) and cryptosporidiosis (43). For the interpretation of the current study, it is likely that drinking water treatment was poorer in areas with animals compared to those without as water treatment in rural areas tends to be worse than urban areas (77). Thus, water treatment may explain some of the results of the analyses that had zero animals as the reference category. However, it is very unlikely that water treatment per se will incrementally deteriorate as animal density increases. Therefore treatment of drinking water is unlikely to explain the results of those analyses where a progressive increase in disease risk was associated with an increase in animal density.

5.4 Discussion of results of ecological analyses

The results of the analyses that included all mainland meshblocks categorised into zero, low, medium, and high animal densities will first be discussed. Subsequently, results from the six category analyses that only included meshblocks with dairy, beef or sheep will be discussed.

5.4.1 Using zero, low, medium and high animal density categories as explanatory variables

This study identified that rates of VTEC and cryptosporidiosis tended to be significantly higher in those areas with any of the three animal types (sheep, beef cattle or dairy cattle) relative to those that have no animals of the respective types, even after adjusting for age, deprivation, ethnicity and animal type. In addition, for some of the reported analyses there was a step up in disease risk when the animal density increased from zero to low; but as density increased disease risk did not continue to increase (for example, in the analyses of the relationship between beef density and both VTEC and cryptosporidiosis).

The step up in disease risk suggests there may have been a factor increasing the risk of VTEC or cryptosporidiosis notifications in areas with animals, but unrelated to the density of animals. For example, poor water treatment in areas with animals. Bias in notification rates is unlikely to explain the step up in risk as those in rural areas (as indicated by the presence of animals) are expected to have poorer access to health care and lower notifications than those in urban areas.

Some of the analyses did not show the same step up in disease risk. For example, dairy density and cryptosporidiosis. Rather, there was evidence that risk of disease increased incrementally as density of animals increased. These findings indicate that the density of selected animal types may explain some of the variation in disease rates.

5.4.2 Using six category animal density categories as the explanatory variables

To explore better whether the density of dairy cattle, beef cattle, or sheep explained the variation in VTEC and cryptosporidiosis rates, a second set of analyses were performed which excluded meshblocks with no animals (by respective type). While these exclusions reduced the numbers of cases available for the analyses, the included meshblocks were more comparable to each other as there was some degree of rurality in all. Therefore these analyses were less likely to be affected by confounding by, for example, water treatment. Six animal categories were used to more reliably investigate a dose-response relationship between animal density and disease. These analyses also had minimal collinearity between variables (correlation coefficients all <0.5 and for animal variables <0.19), which contrasted with the previous analyses which included the meshblocks with no animals. Minimial collinearity not only was likely to have contributed to more complete confounder control, but also the effects of the different animal types on the disease outcome could be more confidently distinguished.

5.4.2.1 Dairy cattle areas

For VTEC, this study identified that increasing dairy cattle density was significantly associated with an increased risk of VTEC within New Zealand. When comparing these results with the three comparable studies performed internationally, the findings of the current study are consistent with only one. Haus-Cheymol et al. in France (90), identified a positive association between dairy cattle density and a surrogate of VTEC, haemolytic uraemic syndrome. In contrast, Michel in Ontario, Canada (85) did not identify a significant association between dairy density and VTEC incidence and Febriani et al., in Quebec, Canada (92) did not identify an association between dairy density and *E. coli* gastroenteritis

hospitalisations. However, it is possible that these studies may have not detected an association between dairy cattle density and disease because of the relatively small numbers of dairy cattle and small numbers of *E. coli* cases that were included in the respective studies.

For VTEC, while the confidence intervals of the individual incidence rate ratios were wide, there was suggestion of a dose response between dairy density and disease. Specifically, rates of disease increased after density was greater than 50 dairy cattle per km^2. However, this dose response was not entirely consistent. In particular, the category just above the reference had higher rates of disease than the subsequent four categories. This lack of consistency in the dose response relationship between dairy density and VTEC may be due to bias, confounding or, as the 95% confidence intervals are wide, random error. Regardless, the increased risk of VTEC in some dairy density categories requires further investigation. This is particularly important not only in the context of the increasing incidence of VTEC and dairy cattle numbers in New Zealand, but also that dairy cattle in New Zealand are known to carry VTEC (69, 122-124), including the 0157 serogroup (122).

For cryptosporidiosis, within dairying areas, there was a clear dose response between dairy density and disease. Incremental increases in dairy cattle density were associated with an increase in cryptosporidiosis notification rates that was highly statistically significant when tested for trend ($p<0.001$). While it may be useful to compare these results with other studies, there has been no published international or local research that have investigated the relationship between dairy cattle density per se and human cryptosporidiosis. The study by Close et al. in Canterbury, New Zealand (91) was the most comparable to the current one. They found cryptosporidiosis rates in dairying areas with 'major irrigation' were 2.1 times higher than those in dairying areas without major irrigation. From their research, it was not possible to determine if this increase in risk was due to dairy density or due to the irrigation type.

The findings of the current study are consistent with dairy cattle density being causally associated with cryptosporidiosis in New Zealand. This is supported by research showing *C. parvum* infects dairy cattle in New Zealand (prevalence of infection is between 0.6% for adult cattle (45) and 21% for calves in spring (40)), and that infected calves excrete vast numbers of oocytes (up to 10^6 oocytes per gram of faeces (40)). New Zealand studies also show dairy cattle *density* is an important predictor of microbial contamination of waterways (as indicated by *E. coli* concentrations) (53, 125). Thus, it is plausible that if animals are carrying

Cryptosporidium and humans are obtaining water from sources contaminated by the animals, then as dairy cattle density increases the risk of cryptosporidiosis also increases in an incremental manner.

This study is unable to confirm the transmission routes by which the relationship between dairy cattle density and cryptosporidiosis is mediated. However, several international cryptosporidiosis outbreaks have been mediated by drinking water contaminated by runoff from land stocked with agricultural animals (58). Therefore water contamination is likely to be important in mediating the relationship between dairy density and disease. Additional support for water contamination being an important mode of transmission is found in research showing *Cryptosporidium* contaminates New Zealand waterways (75, 76). Further New Zealand research by Duncanson et al. has also identified that the risk of cryptosporidiosis was higher in areas with poor quality water supplies compared to those with good quality supplies (125). While it is possible that these findings may be explained by areas of poor quality water supplies also having higher densities of dairy cattle, the current study is unable to confirm this hypothesis as it did not incorporate water zones in the analysis.

5.4.2.2 Beef cattle areas

In contrast to dairy cattle, this study found no statistical association between beef cattle density and VTEC, but did find an inverse relationship between beef density and rates of VTEC. While the test for trend was not statistically significant (p for negative trend = 0.075), the inverse relationship was surprising; especially considering that cattle are important reservoirs for VTEC internationally (11, 36, 127) and that the density of beef cattle has been positively associated with VTEC in some countries overseas (85, 96). Within New Zealand, the importance of beef cattle as reservoirs of VTEC remains uncertain. New Zealand studies that have attempted to isolate VTEC from cattle have either involved dairy cattle only (122, 124), or have not distinguished between beef cattle and dairy cattle (68, 69, 123, 128).

There was also an inverse relationship between beef density and cryptosporidiosis. In contrast to VTEC, the test for trend was highly significant (p for trend = 0.001). This finding is also surprising given that internationally cattle are considered significant reservoirs of *Cryptosporidium* (29, 39). Within New Zealand, as for VTEC, studies of *Cryptosporidium* in cattle have sampled dairy cattle, not beef cattle (40, 45, 70, 71). Therefore, as for VTEC, it is not known whether beef cattle are important reservoirs of *Cryptosporidium* in New Zealand.

Independent of this uncertainty, the trends observed in this study of decreasing disease rates as beef density increased requires further research.

5.4.2.3 Sheep areas

Within sheep areas, this study found no statistical association between sheep density and VTEC risk. This finding was consistent with Valcour et al. in Canada (87) but contrasted with that of a Scottish study which found "sheep density per human population density" to be significantly associated with increasing VTEC incidence (89).

The absence of an association between sheep density and disease in this study appeared to be at odds with international evidence demonstrating sheep are important reservoirs of VTEC (36, 37). However, within New Zealand, while VTEC has been isolated from both sheep meat (68) and in up to 66% of sheep faeces (69), there have been no reports of the 0157 serogroup being isolated from sheep (68, 69, 123). Therefore, it is possible that sheep in New Zealand are more likely to carry non-0157 than 0157. As non-0157 is less likely to be tested for (and therefore notified) than the 0157 serogroup amongst humans in New Zealand (79), it is possible that a true association between sheep and VTEC may have been missed in the current study.

Increasing sheep density was associated with an incremental increase in cryptosporidiosis risk but only once sheep density was greater than the threshold of 100 sheep per km^2. Below this density threshold, there was no relationship between sheep density and cryptosporidiosis. These results contrasted to those of the relationship between dairy density and cryptosporidiosis where there was no density threshold (cryptosporidiosis risk increased incrementally from the lowest dairy density categories). A density threshold in sheep and not in dairy cattle is plausible as sheep are smaller than dairy cattle and produce approximately 15 times less faecal matter (129), and thus if infected with *Cryptosporidium*, are likely to excrete fewer oocytes.

The findings of the present study suggest that sheep density, like dairy density, is likely to be causally associated with cryptosporidiosis, especially once sheep density is greater than 100 sheep per km^2. The findings were comparable to those of Pollock et al. in Scotland, who found sheep density to be significantly associated with cryptosporidiosis rates (86). In New Zealand, despite large numbers of sheep, no studies have been performed that assess the

prevalence of *Cryptosporidium* carriage in sheep (130) thus their role as a reservoir species in New Zealand remains unknown. However, internationally it is well recognised that sheep carry *Cryptosporidium* (mean prevalence 30%) (94); that it causes neonatal diarrhoea in lambs (39); and that *C. parvum* (pathogenic in humans) has been the predominant *Cryptosporidium* species (67%) identified in those studies where genotyping has been performed (94). Therefore, these international studies, when combined with the findings of the current study, suggest that sheep may be acting as an important reservoir of *Cryptosporidium* in New Zealand.

5.5 Implications of research

The descriptive analyses found that the rates of both VTEC and cryptosporidiosis increased over time from 2004 to 2009. While this increase was most prominent for VTEC, it highlights the importance of remaining vigilant in terms of surveillance and prevention of both of these diseases.

The risk of VTEC and cryptosporidiosis was found to be highest in young children. Therefore, attention should be applied to ensure the risk is minimised in this group. Parents and physicians serving rural areas should also be aware of the potentially severe consequences of VTEC infection on children, including haemolytic uraemic syndrome.

The study identified there were higher rates of VTEC and cryptosporidiosis in rural areas relative to urban, as well as in areas with animals relative to those without. It is possible that these results may have been explained in part, by poorer water treatment in rural areas or in areas with animals. Therefore, one mode of prevention could focus on improving water treatment within rural areas.

The ecological analyses identified a significant relationship between dairy cattle density and cryptosporidiosis risk which was very unlikely to be explained by chance, bias or confounding. Given the increasing intensification of dairy farming in New Zealand (72, 131), this study suggests the risk of cryptosporidiosis may increase further as dairy farming intensity increases and more people are exposed to this intensive farming. In this context, the results indicate the need for greater involvement of the public health sector in agricultural decision making including the development of agricultural policy.

While an association was identified between dairy density and VTEC, the results suggested that the relationship was subject to a degree of confounding or bias that could not be assessed in the current study. Regardless, in the context of increasing VTEC numbers and the intensification of dairy farming in New Zealand, the findings indicate the need for ongoing VTEC surveillance and research into the determinants of this disease.

This study also found a significant association between sheep density and cryptosporidiosis risk that was unlikely due to bias, chance, or confounding. While the numbers of sheep may be decreasing in New Zealand (67), this study highlights the need to remain vigilant for cryptosporidiosis in sheep areas, particularly if there is any reversal of the current downwards trend in the intensity of sheep farming.

This study highlighted potential problems with existing animal data collection systems. Firstly, this study highlighted that the Agribase™ database is unlikely to be accurate in detecting future changes of animal numbers over time, especially for dairy cattle[2]. In addition, the Agribase™ data is not easily accessible to public health practitioners such as those within Public Health Units. While Statistics New Zealand data may be useful in describing data trends over time, at a more reasonable cost, most of their agricultural data are largely based on surveys and data resolution is limited to regional council. While there is an exception in the Agricultural Production Census (67), which has a geographical resolution of the census area unit (111), the Census only occurs every five years, limiting analyses of trends over time. As a result of these problems related to accuracy and availability of existing animal data collection, it is possible that associations between animal density changes and zoonotic diseases incidence may go unnoticed in the absence of specific research. Therefore, this study calls for improvements in both the accuracy and availability of agricultural data in New Zealand.

[2] Just prior to this dissertation being submitted in October 2010, Agribase™ identified that the agricultural data on which this research was based had some errors. The new data were re-analysed using the same methods as in this dissertation and the results were not significantly different from those presented here. Therefore, the conclusions remain unchanged.

5.6 Further research

This study identified associations between farm animal density and both VTEC and cryptosporidiosis. These findings warrant further research.

Further research could include improving the modelling of the relationship between animal density and disease risk. Using more sophisticated statistical methods would be an important step. In the analysis for this study, animal density variables were categorised which had the advantage of not forcing a particular relationship between animal density and disease risk, but information was lost with categorisation. Using density as a continuous measure and exploring the relationship using more flexible models should lead to a greater understanding of the relationships between animal density and disease. In the analyses, this study used robust standard errors for the Poisson regression models to allow for departures from an underlying Poisson distribution. However, this is a crude approach, and models which capture the way the data are generated would give more reliable results. In this study, misclassification of animal density was likely and was contributed by the assignment of animals to a meshblock based on farm gate location and not the location of the actual farm. Use of statistical methods which allow smoothing of animal densities would help to more accurately capture the true animal density for each meshblock.

Confounder control in future modelling could be improved by incorporating data on other potential confounders such as water treatment and additional agricultural animal densities such as deer. Future modelling could also examine for interactions between animal density and disease; in particular, it would be valuable to determine whether the increased risk of cryptosporidiosis in dairy areas varies by the age of the cases. Further modelling could also assess whether variables that are likely to be mediators in the relationship between animal density, and VTEC and cryptosporidiosis are spatially correlated. These mediators include rainfall, irrigation and temperature. Modelling could also assess the relationship between changes in animal density over time and the incidence of disease within the same geographical areas and/ or include as outcomes other important zoonotic infections such as campylobacteriosis and salmonellosis. Finally, future modelling should examine whether the relationship between animal density and disease is modified by those farm management practices (such as riparian buffer strips) designed to reduce faecal contamination of waterways (132).

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