Epidemiology of Theileria orientalis in cattle in New Zealand

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Introduction

Until recently, anaemia associated with *Theileria orientalis* in New Zealand was considered to be a sporadic event that only affected cattle debilitated by another condition. In 2009, an outbreak of anaemia due to *Theileria orientalis chitose* strain was reported in naïve animals from the Otago region brought into the Northland region (McFadden *et al.* 2011); the Northland region being known to be endemically affected with the cattle tick (*Haemaphysalis longicornis*), the vector for *Theileria* in New Zealand. Investigation of this outbreak showed that the New Zealand strain of *Theileria* was not necessarily benign and that disease could occur in healthy animals not affected by another agent or condition.

In late 2012 there appeared to be an increase in the number of reports of anaemia (HCT<0.25) associated with evidence of *Theileria* infection in cattle. Over the period 2000 to 2012 there were 12 notifications to the Ministry for Primary Industries (MPI, Wallaceville, New Zealand) i.e. approximately one report per year. For most of these historic reports there was generally only one animal affected and disease caused by *Theileria* was not considered to be the primary condition. From late August 2012 until late April 2013 there were 38 confirmed reports, providing evidence that there had been a change in the epidemiology of *Theileria*. In addition, in some of the affected herds there was a high prevalence of animals with anaemia. These outbreaks tended to follow the same pattern described by McFadden *et al.* (2011); that is, disease in presumed naïve cattle, subsequent to mixing with affected animals. However, this was not the case in all circumstances with anaemia associated with *Theileria* occurring in dairy-cow herds with limited animal movements as well as disease occurring in beef calves that had been born on Northland properties. This is the first time that outbreaks of anaemia associated with *Theileria* have been reported in calves in New Zealand.

In response to the increased number of reports of anaemia, further investigations were carried out. The aims of these investigations were to document outbreaks over 2012/13; determine if there was an emerging disease issue associated with *Theileria*; confirm that reported outbreaks were not caused by an exotic strain of *Theileria*, or that there had been a change in strain type of *Theileria* since the previous large scale investigation in 2009 (McFadden *et al.* 2011); and to gain a greater understanding of the epidemiology of anaemia associated with *Theileria* in New Zealand.

Investigation methods

The diagnosis of the syndrome of anaemia associated with *Theileria* was made by excluding other possible causes of anaemia (Figure 1). Hence the case definition for the *Theileria* anaemia syndrome was "clinical signs in affected animals consistent with intravascular haemolysis (including lethargy/depression, pale/white/jaundiced mucous membranes, fever, possible haemoglobinuria), the presence of a regenerative anaemia (anaemia defined as HCT<0.25; Riond *et al.* 2008) and *Theileria* present on a blood smear in one or more animals and no other biologically plausible reasons for other causes of anaemia". The key differentials for a regenerative anaemia were considered to be copper toxicity, post parturient haemoglobinuria (low phosphorous), leptospirosis, Heinz body anaemia, a high tick burden, or anaemia

caused by *Theileria* secondary to immune suppression e.g. from bovine viral diarrhoea virus.

Figure 1. Diagnostic tree for cattle with clinical signs of anaemia

Where there were one or more animals determined to be anaemic with the presence of *Theileria*, a proportion of cattle from affected cattle herds were tested to determine the herd prevalence of anaemia (and *Theileria* strain type). These prevalence studies were generally carried out on cattle from the same management group; however, in some cases additional management groups were also tested to determine if there were any differences associated with a particular factor of interest.

Whole blood was collected into EDTA. A blood smear was made, suitable for visualising any *Theileria* species present using light microscopy. Blood smears were stained with a Romanowsky stain (May-Grunwald/Giemsa; Technecult Laboratories, Napier, NZ). In addition, the HCT was determined from the blood. The formula for HCT= mean cell volume (MCV) x rbc where the MCV and rbc are measured variables.

Case studies

Three case studies are described that reflect the characteristics of high prevalence outbreaks observed late in 2012.

Case study 1, Northland dairy-cow herd

This dairy herd in Northland consisted of 465 Jersey-Friesian cross cows with a planned start of calving of 22 July 2012. The herd had moved to Northland from the Waikato region in June 2012 where it had been farmed for ten years. Prior to that, it had originated from the Northland region. There were 16 animals that had been born prior to 2002 in the Northland region; the rest were born in the Waikato.

The first indications that *Theileria* was causing disease in the dairy-cow herd was on 22 August 2012 when a single animal was diagnosed with a regenerative anaemia (Reticulocytes=107; HCT=0.08) with the presence of a low number of *Theileria* (count <1 per 1000 rbc). Subsequently two more animals were detected with a regenerative anaemia and *Theileria* present; leading to a sample of the herd being tested.

A sample of 29 cows was selected randomly during milking. Cows milked in a herring bone milking parlour were selected from the first and last part of each row with rows systematically selected from all rows passing through the parlour. Eighty three percent (95% CI 64-94%) of the herd was affected with anaemia at the time of sampling. Confidence intervals were calculated using the epiR package in R v2.15 (R Development Core Team, 2011; R Foundation for Statistical Computing, Vienna, Austria).

The HCT and *Theileria* count indices were monitored by serial blood tests (Table 1). On the final test, carried out eight months after the first, one animal (17%; 1/6) remained anaemic. Generally the same animals that had been previously tested were retested rather than testing from a new selection. Over the early period of the outbreak (31 July until 7 September 2012) 17 cows died; although the exact cause of death was not confirmed as *Theileria*. No significant findings were present on gross pathology from two post-mortems carried out. Histology of tissues collected from one animal showed centrilobular coagulative necrosis of the liver and erythroid extramedullary haematopoiesis. The centrilobular hepatic necrosis was consistent with acute hypoxia due to anaemia. The extramedullary haematopoiesis was consistent with a regenerative response to pronounced anaemia without iron loss. Tissues collected from post mortem examination from the other animal were not suitable for histology.

As part of the investigation, the affected cattle within the dairy cow herd were examined and tested for other possible causes of anaemia. There were no clinical signs suggestive of other chronic conditions that could have caused anaemia. Blood biochemistry for a sick ruminant profile was unremarkable from six animals tested (New Zealand Veterinary Pathology, Hamilton, New Zealand). The burden of ticks on cattle was relatively low and variable between animals. Tick specimens removed from cattle were identified as *Haemaphysalis longicornis* (*Ixoda: Ixodidae*). The tick specimens were confirmed as having *Theileria orientalis* present by PCR.

Serological tests and tests of trace elements on blood from 6 to 10 animals gave no evidence for other potential causes of anaemia. Serum phosphate (1.69mmol/l, reference range 1.1-2.8mmol/l), magnesium (0.82mmol/l, reference range 0.62-1.15mmol/l), and copper (9µmol/l, reference range 8.0-20umol/l) were within normal limits. An ELISA antibody test performed on serum provided evidence of previous exposure to *bovine viral diarrhoea* (BVD) *virus* (HerdChek BVDV Antibody Test, IDEXX Laboratories, Maine, USA); however, BVD viral antigen was not detected using an ELISA test for BVD virus in whole blood in EDTA (HerdChek BVDV Antigen Test, IDEXX Laboratories, Maine, USA). Serology was negative for *enzootic bovine leukosis* (EBL) using an ELISA antibody test (ELISA Bovine Leukosis Serum blocking, Institut Pourquier, Montpellier, France); negative (≤1/50) for *Leptospira interrogans* serovar *Pomona* and *Hardjo* using the microscopic agglutination test (MAT); and negative for Johnes disease using an antibody ELISA. There was one from six animals positive for liver fluke (Fluke ELISA).

On 28 September 2012 between testing events four and five, a treatment trial was carried out using an intramuscular corticosteroid. The trial was designed to test the theory that there had been an automimmune component to the anaemia occurring secondary to disease caused by *Theileria*. Twenty cattle were randomly assigned to treatment (n=8) and control (n=12) groups. The treatment group were injected intramuscularly with 7ml of Voren suspension (Dexamethasone isonicotinate; Boehringer Ingelheim Limited, Manukau City 2016, New Zealand). A sample size of 10 per group was sufficient to detect a difference in HCT of 0.04 with a power of 80%.

After seven days HCT was determined from blood collected from cattle from both groups. Comparisons between

HCT between treatment and control groups were carried out using Student *t* test (base package in R v2.15; R Development Core Team, 2011; R Foundation for Statistical Computing, Vienna, Austria). There was no difference in HCT between the groups (HCT 0.241 for the treatment versus 0.238 for the control, p=0.62), nor was there any difference in the change in HCT for each of these groups over the seven day trial period (change in HCT 0.008 for the treatment versus 0.011 for the control, p=0.62). The trial was repeated on 12 October 2012 between testing events five and six using a higher dose of corticosteroid (Ilium Trimedexil, 5mg/ml dexamethasone trimethylacetate; Ethical Agents, Wiri 2104, Auckland, New Zealand) with similar results (HCT 0.241 for the treatment versus 0.234 for the control, p=0.48) indicating that treatment with corticosteroids at different dose rates did not appear to alter the HCT in the treatment vs. the control groups.

Milk production of the herd was below average for the region for the 2012/2013 season (230kg milk solids/cow compared with 315kg milk solids/cow for the Northland region; Anonymous 2012). Reproduction indices for the 2012/2013 season indicated that reproductive performance had been relatively poor. The empty rate for the herd was 18% for 19 weeks mating (Target 5%). The herd had a three week submission rate of 80% (Target 90%) and a six week in calf rate of 44% (Target 78%). The calving pattern for the previous season had been below target and could have impacted on the 2012/2013 reproduction indices. In addition, drought conditions occurred over the autumn period and are likely to have had some effect. It was not possible to quantify the effect of anaemia on these indices.

	Test 1	Test 2	Test 3	Test 4*	Test 5#	Test 6	Test 7	Test 8
Date tested	22/8/12	31/8/12	11/9/12	26/9/12	4/10/12	19/10/12	29/11/12	11/4/13
Days since anaemia first identified	0	9	20	35	43	58	99	232
Number tested	1	2	29	25	25	23	25	6
Mean HCT (L/L)	0.08	0.17	0.21	0.23	0.23	0.24	0.25	0.28
Standard deviation HCT (L/L)			0.04	0.03	0.02	0.02	0.02	0.03
Median *Theileria* organism count (per 1000 rbc)	<1	78 (0, 156)	1(0, 56)	2(0, 23)	1(0, 34)	<1(0, 27)	<1(0, 8)	<1(0 ,4)

*Treatment trial carried out on eight animals using 7ml Voren suspension 1mg/ml.
#Treatment trial carried out on seven animals using 6ml Trimedexil 5mg/ml.

Table 1. Haematocrit (HCT), and Theileria organism counts/1000 red blood cells determined using light microscopy for EDTA whole blood collected from cattle on the affected Northland property over time

Case study 2, Waikato cattle herd

The affected dairy-cow herd was located at Taupo in the Waikato region and comprised three main management groups of cattle. The first group were born and bred in the Waikato and were either in calf or recently calved and in the milking herd (r). The second group were milking cows that had been brought-in from another property from the Waikato but were not in calf (w). The third group were animals from Northland which had entered the milking herd five months prior to the first anaemic animal being noticed on the property (n).

The first clinical case was observed in a cow from group 'r' that had originated from the Waikato. The affected cow was lethargic and subsequently aborted. Ten days later, six more clinically affected animals were identified from the same group. All six animals had *Theileria* organisms present on blood smears and were mild to moderately anaemic. Over the subsequent few days one of the six affected animals died. A necropsy was carried out and tissues collected for histology. The findings of these examinations were consistent with death due to acute anaemia. At the same time six apparently normal animals were sampled from the milking herd and all were found to have *Theileria* organisms present on blood smear. Two of these animals originating from Northland (group n) had a normal HCT, but three of the four from group 'r' originating from the Waikato had an HCT below the reference range despite being clinically normal on examination.

An investigation was carried out to try and understand any differences in anaemia between the three groups. Blood was collected from 15 cattle from each of the three groups and blood smears examined for counts of *Theileria* organisms (Figure 2).

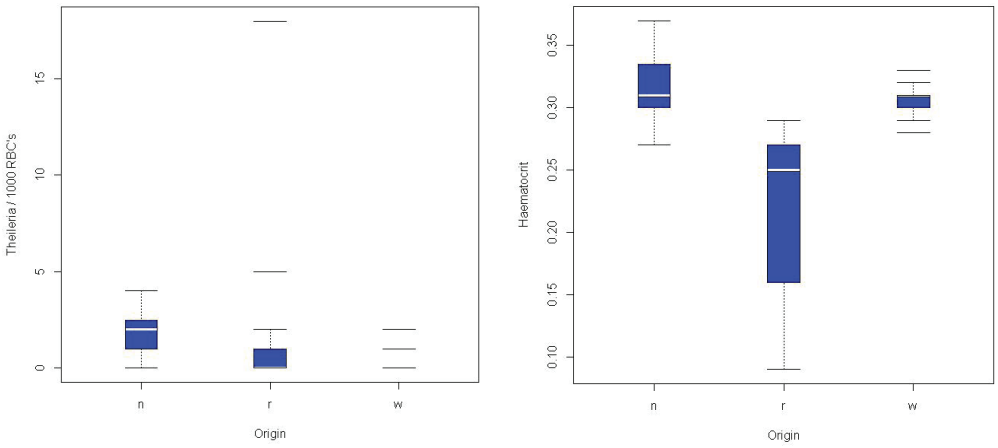


Figure 2. Boxplot of Theileria count per 1000 red blood cells and haematocrit from a sample of cattle tested from three management groups (r = Waikato origin; w = not in calf Waikato origin; and n = Northland origin)

Additional testing was performed excluding other causes of anaemia including copper, phosphate, magnesium and leptospirosis. In the month following this cross sectional blood sampling, no further animals became clinically ill or died. We hypothesised that animals from Northland, where *Theileria orientalis* is known to be endemic, transferred *Theileria* either by ticks or by iatrogenic means to the naïve Waikato population of cattle. Whilst ticks may have been the vector for transfer of *Theileria* between cattle groups there had been no observations of ticks on cattle.

The naïve animals that were under physiological stress from calving had a lower HCT than either the cattle of Northland origin or those that were of Waikato origin and were not in calf. Individuals from each of the three groups had low numbers of *Theileria* present on blood smear. During this outbreak one animal died and nine of twenty five tested animals (9/25, 36%) from the stressed naïve group were anaemic.

Case study 3, Beef calves Northland

In a group of 45 three-month-old Hereford-Friesian-Limousin cross suckler calves (still on dams), two calves were observed to be anaemic, lethargic, and haemaglobinuric (red water) in early October 2012. Icterus developed about a week later. Over a three week period, one deteriorated and died, while the other recovered. Two other calves developed a similar presentation and died within one week. One further calf was affected and notified to MPI. This calf had an HCT of 0.06 (severe anaemia), and *Theileria* were observed in a blood smear. Negative results were received for *Leptospira Pomona* and BVD antigen. There were approximately 900 adult cattle on the farm and 450 other calves; however, none of these animals were affected. Ticks had been commonly observed on the cattle.

Epidemiology

Data from cases of anaemia associated with *Theileria* reported to MPI between August 2012 and April 2013 were analysed for the purposes of understanding the broad trends of outbreaks. On some farms cross sectional sampling of multiple animals was performed and herd prevalence calculated (Table 2). On other farms, only single animals were sampled.

Haematocrit and theileria count results

Haematocrit and *Theileria* counts were carried out on 332 individual animals from multiple farms (38). In some cases

individual cattle were tested multiple times with a total of 422 tests carried out. Of the 332 animals tested 129 (39%) had anaemia confirmed in one or more tests.

Comparisons between HCT and *Theileria* count were carried out using the Wilcoxon rank sum test with continuity correction. Animals were classified as anaemic with an HCT <0.25 and non-anaemic where HCT ≥0.25. Analysis showed that animals that were anaemic were more likely to have had a higher *Theileria* count than those that had a normal HCT (p <0.01; Anaemic animal *Theileria* count per 1000 rbc = 2 vs. <1).

Prevalence studies, where testing was carried out on five or more animals, were completed on 17 farms. In 6 of 17 farms (35%) the median HCT from individual prevalence studies was less than 0.25 (mean = 0.26, Min=0.12, Max=0.35). On those herds tested there were generally a greater number of anaemic cattle on dairy farms than in beef herds (Figure 3). The prevalence was quite variable between affected farms. The mortality in affected herds was generally quite low (1%), (Table 2).

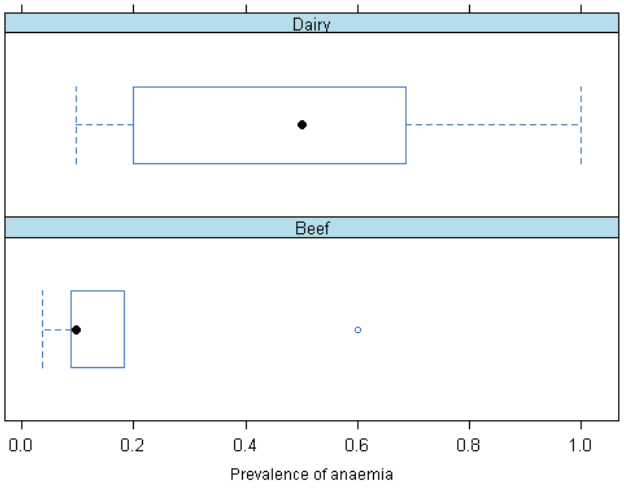


Figure 3. A boxplot of the proportion of cattle with anaemia (HCT<0.25) from 17 beef (calves) and dairy-cow herds (mixed age cows) herds where prevalence studies were carried out. All herds had met the case definition for the syndrome of anaemia associated with Theileria orientalis

Unique database ID	Date notified	N dead	N herd	N anaemic	N tested	Prevalence of anaemia with 95% CI	Region	Farm type
30	30/08/2012	1		12	56	21% (12-34%)	Waikato	Dairy spring calving
27	7/09/2012	20	467	39	46	85% (71-94%)	Northland	Dairy spring calving
38	20/09/2012	1	88	1	11	9% (0.2-41%)	Northland	Beef
40	20/09/2012	1	60	3	31	10% (2-26%)	Northland	Dairy spring calving
31	1/10/2012	0		2	23	9% (1-28%)	Northland	Beef
26	25/10/2012	0	300	1	27	4% (0.1-19%)	Northland	Beef
39	31/10/2012	2	130	1	10	10% (0.3-45%)	Northland	Beef
94	8/11/2012	0		3	5	60% (15-95%)	Northland	Beef
46	12/12/2012	0	500	1	6	17% (0.4-64%)	Northland	Dairy town supply
81	20/03/2013	0	304	2	12	17% (0.4-64%)	Waikato	Beef
93	27/03/2013	1	150	6	6	100% (42-100%)	Waikato	Dairy town supply
95	5/04/2013	0	50	2	11	18% (2-52%)	Waikato	Dairy town supply
87	9/04/2013	0		3	9	33% (7-70%)	Bay of Plenty	Dairy spring calving

CONTINUED...

Unique database ID	Date notified	N dead	N herd	N anaemic	N tested	Prevalence of anaemia with 95% CI	Region	Farm type
88	10/04/2013	3	45	12	23	52% (31-73%)	Auckland	Dairy town supply
89	11/04/2013	3	20	10	19	53% (29-76%)	Auckland	Dairy town supply
97	16/04/2013	13	270	7	8	88% (47-99%)	Waikato	Dairy spring calving
98	16/04/2013	1	250	6	12	50% (21-79%)	Auckland	Dairy spring calving

Table 2. Table of the percentage of cattle tested with anaemia (HCT<0.25) from 17 herds (beef calves or young stock from beef herds and mixed age cows from dairy-cow herds) where prevalence studies were carried out. All herds had met the case definition for the syndrome of anaemia associated with Theileria orientalis

Analysis of strain types

Herds tested for *Theileria* were classified into four groups. Analysis of *Theileria* strain types were carried out on EDTA whole blood or serum from cattle from the four groups. The four groups were:

1. Outbreak properties 2012-13; where outbreaks fitted the case definition provided and samples were available (number of herds = 20).
2. Historic outbreaks in cattle herds pre 2012; *Theileria* DNA stored from four previous investigations from 2009 and 2011 and three from pre 2009 (McFadden *et al.* 2011), (number of herds = 7).
3. Non disease reports; where *Theileria* had been identified on a blood smear, but the case definition was not met (i.e. there was no evidence of anaemia), (number of herds = 11).
4. Surveillance samples (collected for the purposes of arbovirus surveillance); including serum collected from cattle from Northland that did not necessarily have either anaemia or *Theileria* present in the blood. Serum was tested from pooled samples collected from approximately 12 cattle per herd to give the overall strain type for that herd. The sensitivity of detecting *Theileria* in serum is likely to be less than when testing whole blood; however only serum was available. Samples were available from multiple herds for the period 2008-end of 2012. The availability of the surveillance samples informed on the strain types that had been present in Northland pre 2012 (number of herds = 35).

Theileria orientalis Chitose was the only strain detected in the seven historic outbreaks where stored DNA was available. Samples from cattle surveillance samples (Group 4) were generally carried out once per year with between 5 and 6 herds tested per year. In 2012 cattle were tested twice, once in June and once in December. All samples were negative for *orientalis Ikeda* until the December 2012 sampling when it was detected (Figure 4). The absence of *Theileria orientalis Ikeda* strain in surveillance samples prior to 2012 supports a hypothesis that *Ikeda* was not present rather than simply not detected before 2012.

A generalised linear model (GLM) was used to examine the association of the binomial outcome variable of the occurrence of an outbreak (Group 1 from above) or not (Group 3 from above) with the occurrence of *Theileria orientalis Ikeda* strain. There were variable numbers of samples strain typed per herd from these two classification groups, with most non-disease report herds having only one sample strain typed vs. outbreak properties where there was a generally a number of samples strain typed. Therefore, to account for clustering of strain types within herd, analysis was carried out by randomly selecting one strain type analysis per herd. The odds of *Theileria orientalis Ikeda* strain being present in outbreak herds was greater than in non-disease report herds (Odds ratio = 15, 95% CI = 3-131).

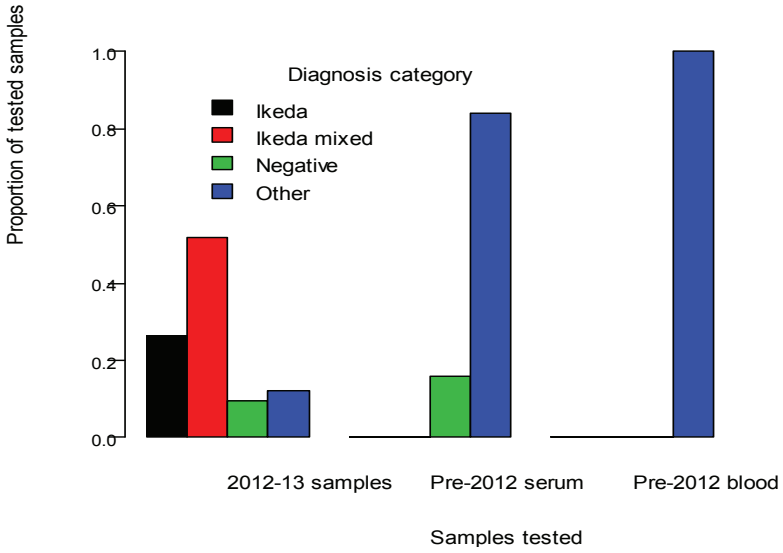


Figure 4. Molecular Theileria strain types detected from pre 2012 and post 2012 whole blood or serum samples

Clustering of cases

Cases were present on 24 dairy farms and 14 beef farms (as of 28 April 2013). Generally calves (one yearling herd affected) were the only age group affected on beef farms. On dairy-cow herds mixed aged cows were affected. Outbreaks of anaemia on dairy farms in the autumn mostly occurred in spring calving dairy-cow herds outside the period of parturition (13/18; 72%). There was temporal clustering in the autumn and spring period (Figure 5) similar to the temporal pattern described in Australia. Most of the recent outbreaks (autumn period) have been in dairy-cow herds in contrast to the end of 2012 when most outbreaks were in beef herds (spring period).

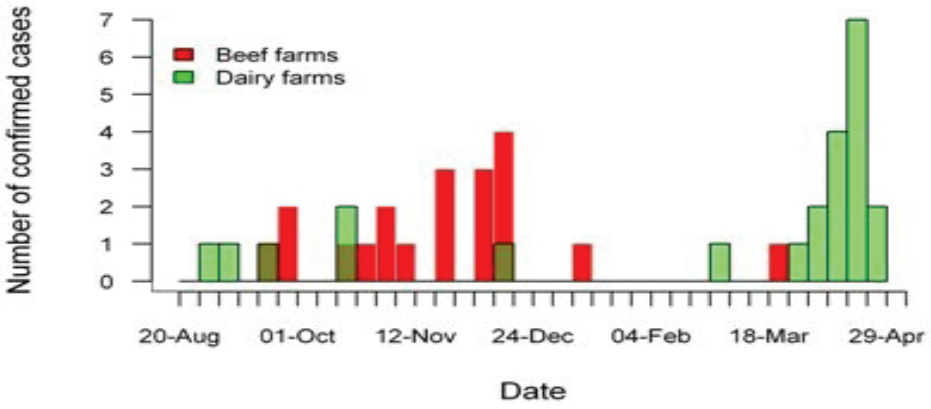
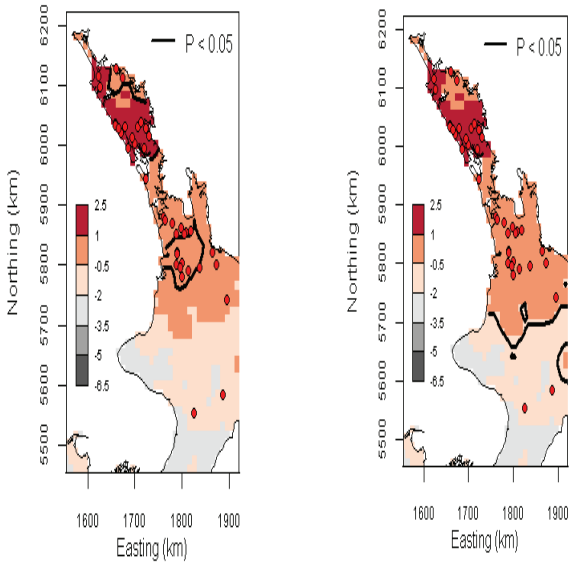


Figure 5. Epidemic curve of report cases of anaemia associated with Theileria orientalis over time for the period August 2012 until April 2013

Point locations of affected cattle farms were added to the spatial risk map shown in Figure 6. Outbreak cases were clustered in Northland, South Auckland and the Waikato. The spatial location of farms was based on the farm centroid and was obtained from farms on line data (http://farmsonline.maf.govt.nz/). Kernel-smoothed relative risk (edge-corrected, adaptive (log) relative risk) maps were produced in the sparr package (Davies *et al.* 2011) in R. Maps give the spatial variation in the risk of a cattle herd being affected with anaemia associated with *Theileria orientalis*.



(A) Areas of elevated risk (B) Areas of decreased risk

Figure 6. Outbreaks of anaemia associated with Theilieria orientalis in cattle herds ( ) over the period end of August 2012 until end of April 2013. The areas where risk was statistically greater (A) or less (B) than other areas (p<0.05) is marked by the black contour line

Discussion

Anaemia can be a vague clinical sign with clinically anaemic animals not necessarily showing obvious signs of disease. Hence anaemia can be difficult to diagnose without the use of laboratory tests. A herd diagnosis may not necessarily be made even when there has been a high proportion of the herd affected. In the 17 prevalence studies generally only one affected animal had been identified and yet prevalence of disease in those affected herds was significantly higher. Whilst a diagnosis of anaemia associated with *Theileria* did occur in the two dairy case studies described it is possible that similar sized outbreaks have occurred but remained undiagnosed. Therefore it is likely that the number of outbreaks reported to the Ministry for Primary Industries underestimates the true prevalence of affected herds.

The majority of outbreaks of anaemia associated with *Theileria orientalis* were clustered in time and space in the Northland, Auckland and Waikato regions between late 2012 and early 2013. The location of outbreaks may in part reflect the presence of the tick vector in these locations (Heath 2013). However, it is possible that other factors have influenced the spatial clustering observed. The hypothesised alternative vector *Stomoxys calcitrans* is active in the same areas where outbreaks have occurred, with peak activity occurring between January and May (Heath 2002).

Generally no agents or conditions other than *Theileria* that cause anaemia in cattle were identified from investigation of outbreak properties. In individual cattle tested, there was a direct association between the level of anaemia and *Theileria* count, providing evidence that *Theileria* was related to the cause of anaemia. This illustrates a change in epidemiology of *Theileria* in NZ where the organism has historically been regarded as non-pathogenic. Further examination of the strain types present in the samples was performed.

One of the strains of *Theileria orientalis* identified by molecular analysis (*Theileria orientalis Ikeda* strain) was the same strain determined to have been responsible for recent outbreaks of anaemia (associated with *Theileria orientalis*) in cattle in Australia (Earmens *et al.* 2013). As had occurred in New Zealand, the *Theileria orientalis* strains identified in Australia were considered to be largely non-pathogenic prior to these reported outbreaks (Izzo *et al.* 2010; Kamau *et al.* 2011; Earmens *et al.* 2013).

Analysis indicated that there was a greater likelihood of *Theileria orientalis Ikeda* strain (identified from New Zealand outbreaks and present in Australia) being present in cattle from herds that had outbreaks of anaemia in comparison to

non-diseased herds. The *Theileria orientalis Ikeda* strain identified from outbreaks was different from strains identified from historical testing for *Theileria* organisms present in New Zealand cattle prior to 2012 (historical investigations and arbovirus surveillance samples). Hence, the current working hypothesis is that the strain identified from recent outbreaks has not necessarily been present in New Zealand cattle in the past.

There were a number of herds identified with *Theileria orientalis Ikeda* strain where no disease was detected. Given the subtleties of anaemia it is possible that disease had not been detected in these herds either because herds were sampled at a time when anaemia had not yet occurred, or subsequent to their recovery; or because insufficient numbers of animals were sampled from the herd to detect anaemia. Regardless of whether disease occurred or not, it is likely that the cause of anaemia was multifactorial and factors additional to the presence of *Theileria orientalis Ikeda* strain were involved. Given that outbreaks have occurred around the time of parturition in a number of dairy-cow herds in the spring and autumn it is likely that environmental stress factors have played a part in the occurrence of disease in some instances. However, a number of outbreaks occurring in the autumn were in spring calving dairy-cow herds indicating that disease could be initiated without the requirement of stress from parturition. Other factors such as tick abundance specific to environmental conditions for 2012/13 may also be associated with temporal clustering.

In the first case study presented on a Northland dairy cow herd there was a very high prevalence of cows affected with anaemia. The cattle tick is a three host tick and therefore a single tick carrying *Theileria orientalis Ikeda* strain can only infect one animal per stage. Hence a significant proportion of the tick population would need to be carrying *Theileria orientalis Ikeda* strain for a high incidence of disease to occur within a short time frame. Alternatively, infection of cattle with the Ikeda strain could have occurred at a slow rate and disease only precipitated by a stress event such as calving. Another possibility is that there are other pathways of transmission occurring resulting in an apparent rapid transmission rate within an affected herd. Iatrogenic spread of *Theileria* is known to occur by transfer of infected blood from one animal to another (Uilenberg *et al.* 1985). The efficiency of spreading *Theileria* by subcutaneous vaccination is not known; however, at face value it would appear to be low to negligible given that only minimal (if any) blood would be transferred between animals by vaccination. An alternative hypothesis is that the stable fly (*Stomoxys calictrans*) could act as a vector for *Theileria*. *Theileria* has been detected in the stable fly (Hadi and Al-Avery 2012); however no studies have been carried out on the role (if any) of the stable fly as a vector for *Theileria*.

In the first case study herd described, serial blood tests showed that there were still anaemic animals in the herd eight months after anaemia and *Theileria* had first been detected. It is likely that there have been significant impacts on production on this farm; however, it is difficult to partition these impacts as being a direct result of anaemia caused by *Theileria orientalis*. We hypothesised that there could have been other underlying disease that could have slowed development of immunity; however, none was detected. Further research is necessary to determine if prolonged anaemia is a feature of other outbreaks of disease caused by *Theileria*. Future efforts need to focus on gaining understanding of the epidemiology of this syndrome in the New Zealand context so that any impacts can be minimised in the future.

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