# Surveillance for *Theileria* associated bovine anaemia (Ikeda) in New Zealand

ANDY MCFADDEN, DAAN VINK, DAVID PULFORD, KEVIN LAWRENCE, PAUL BINGHAM, EDNA GIAS, ALLEN HEATH, JONATHAN WATTS, ROBERT SANSON
Ministry for Primary Industries, PO Box 2526 Wellington

**MPI** – Ministry for Primary Industries
**HCT** – Haematocrit
**TABA** – Theileria associated bovine anaemia

## Introduction

In late 2012 outbreaks of *Theileria* associated bovine anaemia (TABA) were reported in dairy and beef cattle herds (McFadden *et al.* 2013, Lawrence *et al.* 2013). Strain typing of *Theileria orientalis* was carried out on samples collected from cattle herds experiencing outbreaks. One of the strains was identified as *T. orientalis* Ikeda type. Analysis of data from the New Zealand outbreaks showed that there was a greater likelihood of *T. orientalis* Ikeda type being present in cattle from herds experiencing outbreaks of anaemia compared to non-outbreak herds. In addition, individual animals within an affected herd were more likely to be anaemic if the Ikeda type was present compared with animals with endemic strains of *T. orientalis* such as the Chitose type (McFadden *et al.* 2013, Lawrence *et al.* 2013).

Since the initial diagnosis of TABA the Ministry for Primary Industries (MPI) has continued to monitor the New Zealand epidemic through various surveillance initiatives. Monitoring has been important to inform on risk, and understand the epidemiology and impact of the disease. This paper aims to describe methods of past and current surveillance, the spatial and temporal patterns of outbreaks that have occurred over the course of the epidemic, and resulting inferences made.

## Surveillance methods

### Case reports

Initially, surveillance of TABA (Ikeda) involved veterinarians and veterinary pathologists reporting suspect outbreaks to MPI using the 0800 hotline for exotic and emerging disease. Investigation of these early cases usually involved a standard battery of tests to exclude other causes of regenerative anaemia, carrying out studies to determine the intra-herd prevalence of anaemia and risk tracing to determine any association with other infected farms (McFadden *et al.* 2013).

In late 2013, as the number of cases increased, the capacity of the Animal Health Laboratory (MPI, Wallaceville, NZ) to service farmers and veterinarians by testing blood samples for *T. orientalis* Ikeda was stretched. As a result, the technology to carry out the PCR was transferred to the private regional laboratories (New Zealand Veterinary Pathology, Hamilton, NZ and Gribbles, NZ). A subsidy from MPI to the regional veterinary laboratories was instituted for the cost of this test where specific criteria for a suspect case were met. A suspect case was defined as where one or more clinically affected cattle had a haematocrit (HCT) of less than or equal to 0.24 and theilerial piroplasms were observed in a blood smear. The primary objective of the subsidy was to ensure the cost of testing wasn't a barrier for sample submission and in doing so was aimed at increasing both the sensitivity and specificity of surveillance for TABA (Ikeda). At the same time AsureQuality (Auckland, NZ) was employed to collect data related to the affected farm, including the spatial location and the type of farm affected e.g. beef, dairy or dry stock. A standardised analysis (epidemic curve, spatial mapping of cases) was carried out and reported to disease control experts and field veterinarians. This surveillance strategy was revised for the North Island in January 2015 as the disease became increasingly widespread, and the value of identifying specific cases diminished. In addition, as general exposure of herds to the Ikeda type increased, finding the presence of Ikeda in cattle with clinical signs of anaemia was no longer specific to cattle affected by TABA (Ikeda).

The current strategy for the North Island now involves analysing syndromic data from regional veterinary laboratories where case submissions from veterinarians to the laboratory include an indication that affected cattle had anaemia as part of the presenting syndrome, i.e. cases not necessarily confirmed as being TABA (Ikeda) by PCR. Data was removed from analysis where a specific aetiological diagnosis had been reached i.e. anaemia due to Johne's disease; however, the majority of data did not have an associated aetiological diagnosis. Thus it is likely that the specificity of surveillance has diminished (the number of false positives increased) using this form of syndromic surveillance. Conversely the sensitivity of surveillance is likely to have increased (as a result of capturing data from more cases of TABA (Ikeda) where a PCR was not necessarily carried out).

Prior to cases of TABA (Ikeda) occurring in the South Island in late 2014, data did not indicate that *Haemophysalis longicornis*, the vector for *T. orientalis*, was present in regions south of the Tasman and Marlborough regions. Thus, when a small number of cases were reported outside this geographical range (McFadden *et al.* 2015) we considered it important to identify precisely where and when future cases were occurring so as to inform on risk in this part of New Zealand. Thus, the MPI subsidy has continued for all cases fitting the case definition described, that occur in the South Island.

The identification of the cases in the South Island prompted further assessment of the current known distribution of *H. longicornis* in the South Island. A survey relating to the presence of ticks in the South Island was provided to all members of the New Zealand Veterinary Association (NZVA) by email. Veterinarians were asked to submit tick specimens for confirmation of species, as well as data on location of the find and the species of animal the specimen was found on. The survey mainly targeted deer as a sentinel species because of: the timing of de-velveting with ideal climatic conditions for tick seasonal activity, the ease of examination of stags whilst they were being restrained for de-velveting, and pasture management conditions of deer often being more suitable for ticks then those of other species. Response was relatively poor, although reliable (veterinarian). First-hand reports of tick sightings at two localities on the West Coast around Hokitika and Camerons have been received, in addition to the two confirmed records near Murchison and Seddon respectively. Passive reporting as a result of heightened awareness of TABA resulted in a tick specimen from beef cattle reported from Kaikoura being positively identified as *H. longicornis*. Another find occurred from a household in the Rolleston area of Christchurch, reported as part of the Brown dog tick biosecurity response.

## Regional herd exposure

In addition to the surveillance described above, to further understand exposure of cattle herds to *T. orientalis*, pooled serum samples from cattle herds that had been collected historically for the first six months of 2013 and 2014 were tested by PCR for Ikeda (McFadden *et al.* 2013b). This provided an indication of risk to both naive cattle transported into specific regions and to resident cattle already present in those regions.

## Bovine haemoplasmas

Surveillance from case reports identified a small percentage of cases where the TABA (Ikeda) was suspected based on the case definition but not identified by PCR (<7%). As a result investigation was carried out for the bovine haemoplasmas as an alternative cause of anaemia in cattle.

# Spatial and temporal patterns of disease

## Epidemic curve

In broad terms the key features of the epidemic (Figure 1a) have been:

1. The number of cases has been steadily increasing over time.

2. There are two seasonal peaks in case numbers, that in autumn and spring; with the spring peak being the greatest. These coincide with nymph and adult tick activity peaks respectively.

3. Most of the cases detected have occurred in dairy rather than beef cattle and dry cattle (e.g. R1 and R2) herds.

4. Prior to TABA (Ikeda) being detected in late 2012, syndromic anaemia in cattle was relatively uncommon with approximately 11 cases detected per month for 2010 and 2011.

5. There was a close relationship between the number of syndromic reports of anaemia and the number of confirmed cases of TABA (Ikeda), ($P<0.01$, Adjusted R-squared= 0.74; Figure 1b).

6. There was no indication that a significant number of cases were occurring in the South Island (Figure 3).

At a regional level (Figure 2):

7. The Waikato has been more significantly impacted than other regions with regard to the number of outbreaks occurring.

8. The epidemic appears to have increased in spring 2014 compared to 2013 in some of the less affected regions (East Cape, Manawatu-Whanganui, Taranaki, Bay of Plenty) versus Northland and Auckland.

9. In spring 2014, the epidemic extended south into areas that were previously considered to be of low tick risk.

10. There appeared to a large number of unconfirmed cases during the spring 2013, i.e. the difference between confirmed cases and unconfirmed cases (unconfirmed cases being determined from syndromic surveillance).

11. The highest proportion of unconfirmed cases in relation to confirmed cases was in Auckland.

12. The disparity between unconfirmed and confirmed cases appeared to lessen over time; perhaps indicating that the sensitivity of surveillance was increasing.

13. Relatively few cases have occurred in regions in the bottom half of the North Island.

14. Sporadic cases have started to occur in the South Island during spring 2014.

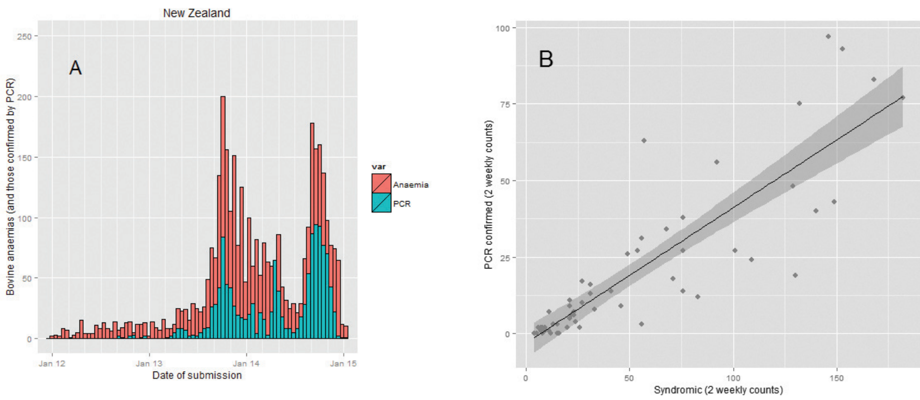


**Figure 1a**. Epidemic curve for confirmed ('Ikeda PCR positive' for the period August 2012 to December 2014) and unconfirmed ('Anaemia syndrome' for the period January 2010 to January 2015) TABA (Ikeda) in cattle for New Zealand as a whole. **Figure 1b**. Correlation between the numbers of cases confirmed and unconfirmed at two weekly intervals over the course of the outbreak (99% confidence interval for the regression line indicated by the dark shaded area).

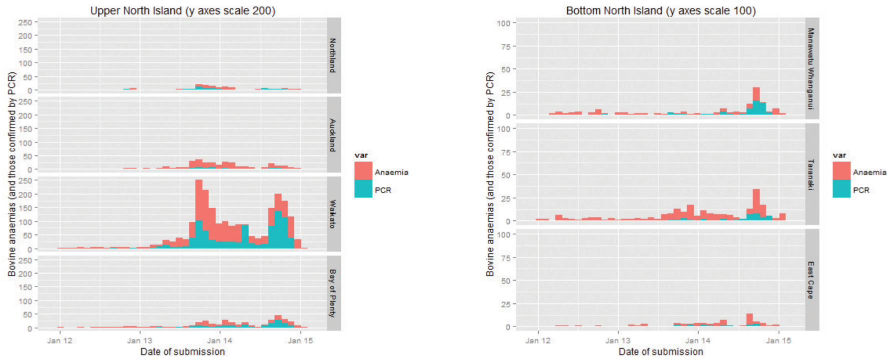


**Figure 2.** Epidemic curve for confirmed ('Ikeda PCR positive' for the period August 2012 to December 2014) and unconfirmed ('Anaemia syndrome' for the period January 2010 to January 2015) cases of TABA (Ikeda) in cattle herds for specified North Island regions (Northland, Auckland, Waikato, Bay of Plenty, Manawatu-Whanganui, East Cape and Taranaki). NOTE: The y axes scale for the Upper North Island regions is 250 compared to 100 for the Lower North Island regions

## The South Island

*Theileria orientalis* (Ikeda) associated bovine anaemia was first confirmed in a 188-cow dairy herd located on the West Coast of the South Island during October 2014 (McFadden *et al.* 2015). The only previous South Island case was in a dairy cow in the Canterbury region in April 2013 (Lawrence *et al.* 2013). The affected cow from Canterbury had been associated with a shipment of cattle from the Hawkes Bay. The data at the time did not conclusively show that Ikeda was being spread by a tick vector in the region, first because ticks had not been found on the farms and, second, knowledge at the time excluded the tick as being present in Canterbury.

Since these first cases, there have been sporadic confirmed cases and several suspect cases in areas outside of the previously known distribution of *H. longicornis* (Figure 4). While ticks were considered to be present in the Marlborough/Golden Bay area, there is substantial uncertainty about their distribution. The response to the tick survey was relatively poor with eight respondents; however, the survey did identify *H. longicornis* in two areas outside of its currently known distribution (Figure 4). Passive reports also indicated a further two locations. This suggests that there are likely to be as yet undiscovered localised tick populations in the South Island. The key question is how far south these may extend.

It is not clear what the future outcome may be for cattle herds in the South Island. The impact on affected farms is likely to be related to the level of cattle exposure to ticks infected with *T. orientalis* Ikeda. Any future impact of the disease on farms in the South Island will relate to how easily ticks establish (even temporarily) and/or are present in local areas of the South Island (south of the Tasman and Marlborough regions).

We were unable to detect ticks on the affected farm in the West Coast or the farm where cattle had been grazed overwinter in Canterbury. It is not clear from our investigation if the tick population on one of these properties can be sustained through multiple years. While ticks have established for a short period in the southern parts of the South Island before, large parts of the South Island do not have a climate entirely suitable to sustain persistent established tick populations. Regardless, even short term establishment for one or two seasons can have an impact. Further understanding of where ticks are present will enable a greater understanding of the risk of TABA.

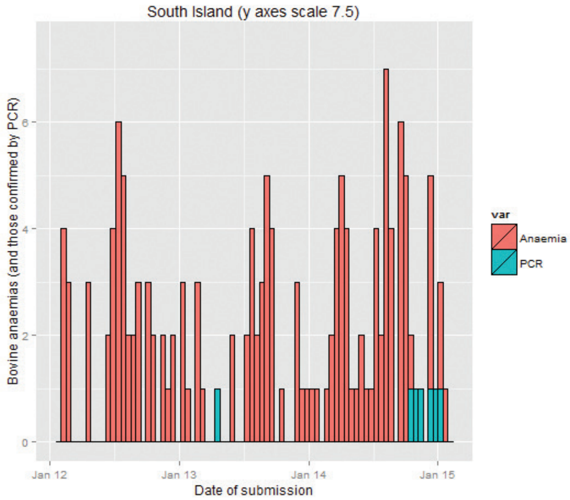


**Figure 3.** Epidemic curve for confirmed ('Ikeda PCR positive' for the period August 2012 to December 2014) and unconfirmed ('Anaemia syndrome' for the period January 2010 to January 2015) cases of TABA (Ikeda) in cattle herds from the South Island. NOTE: The y axes scale for the South Island regions is 7.5

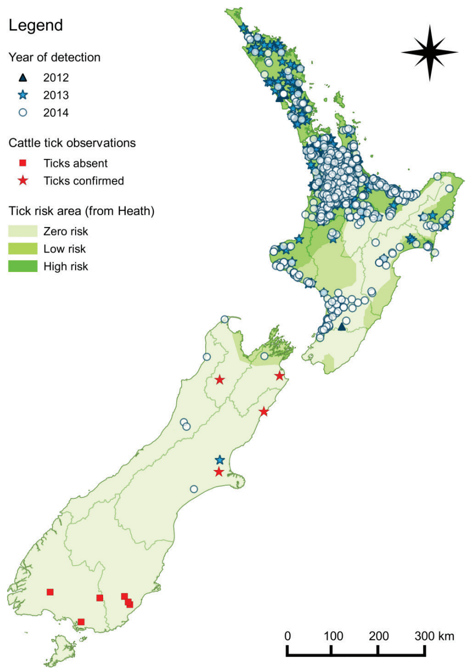


**Figure 4.** Spatial distribution of properties affected by Theileria associated bovine anaemia (Ikeda) in New Zealand. The North Island cases represent cases from August 2012 until December 2014. The South Island cases are those cases from August 2012 until current (March 2015). Ticks detected outside of previous known distribution are indicated

## Herd exposure by region

Data from testing the 2013 and 2014 (Table 1) samples showed that cattle herds present in parts of Northland, Auckland and the Waikato regions had the highest prevalence of herds infected with Ikeda. Gisborne and Hawkes Bay (East Cape), Auckland, Waikato and Bay of Plenty were identified as regions where there was a high percentage of herds exposed to non-Ikeda types only.

The high levels of Ikeda detected in cattle herds in Northland, Auckland and the Waikato regions represents risk to naive cattle being introduced into these regions. There is also the potential for resident cattle herds in the East Cape, Auckland, Waikato and Bay of Plenty to experience future impacts from Ikeda.

Repeat sampling showed that the prevalence of herds infected with the Ikeda had steadily increased over the year in all of the North Island regions where herds were tested. However, there still remained a significant percentage of herds where non-Ikeda types only were present indicating that these herds were at risk of future Ikeda outbreaks. For the East Cape there had been a noticeable increase in the percentage of herds infected, yet with only a small increase in the number of outbreaks reported from the previous year. Thus, presumably outbreaks have gone unobserved or haven't been confirmed by testing. In this region a high proportion of beef herds, where farming is more extensive in comparison to dairy farming could be one explanation.

| North Island Region description | Prevalence *Theileria orientalis* (All types) 2013[2] | Prevalence *Theileria orientalis* (All types) 2014[3] | Prevalence Ikeda type 2013 | Prevalence Ikeda type 2014 |
|---|---|---|---|---|
| Northland | 97% (34/35) | | 94% (33/35) | |
| Auckland and Waikato | 55% (105/191) | 83% (53/64) | 33% (63/191) | 63% (40/64) |
| Bay of Plenty | 34% (10/29) | 43% (6/14) | 10% (3/29) | 21% (3/14) |
| Gisborne and Hawkes Bay | 35% (18/52) | 55% (11/20) | 12% (6/52) | 40% (8/20) |
| Taranaki | 14% (9/64) | 26% (7/27) | 6% (4/64) | 14% (3/27) |
| Manawatu and Whanganui | 5% (5/100) | 22% (13/58) | 1% (1/100) | 16% (9/58) |
| Wellington and Wairarapa | 2% (1/47) | 9% (1/9 ) | 0% (0 ) | 9% (1/9 ) |

[1]based on testing serum samples pooled from approximately 20 cattle sera per herd. Surveys were carried out on samples collected from January to June 2013 and for the same period in 2014. The sensitivity of the test used in 2014 was greater for detecting all *T. orientalis* strains than in 2013 (Hence the increase in apparent prevalence of *T. orientalis* in 2014).
[2]HRM PCR used for the 2013 survey.
[3]Multiplex Taqman PCR used for the 2014 survey.

**Table 1.** Prevalence of cattle herds exposed to all strains of *Theileria orientalis*, and the Ikeda type by regional groups[1]

# Bovine haemoplasmas

A proportion of samples submitted to MPI that fitted the case definition for suspected TABA (Ikeda) yet were negative to *T. orientalis* (Ikeda) were tested for bovine haemoplasma species (samples from 47 herds). The results showed that bovine haemoplasmas were relatively common in the samples tested, with 13% (6/47) and 28% (13/47) of samples with Mycoplasma wenyonii and Candidatus Mycoplasma haemobos present as the predominant species, respectively. The haemoplasmas were present in blood from cattle herds widely distributed across New Zealand. Whilst bovine haemoplasmas have been implicated as causing anaemia, there did not appear to be a greater number of herds with these agents present exhibiting unexplained anaemia, 33% (6/20), (i.e. where anaemia was present without the detection of T. orientalis) when compared with negative control herds, 56% (10/18, non-diseased herds) and herds experiencing outbreaks of TABA (Ikeda), 33% (3/9). Thus, the presence of bovine haemoplasmas in blood does not establish causality for anaemia in cattle. Diagnosis would require exclusion of other causes of regenerative anaemia and an association of the agent with anaemia in affected cattle herds.

# References

**Lawrence K, Mcfadden A, Pulford D.** *Theileria orientalis* (ikeda) associated bovine anaemia: The epidemic to date. *Vetscript* 26 (10), 12-13, 2013

**McFadden AMJ, Pulford D, Lawrence K, Frazer J, van Andel M, Donald J, Bingham P.** Epidemiology of *Theileria orientalis* in cattle in New Zealand. *Proceedings of the Society of Dairy Cattle Veterinarians Annual Conference*, 207-217, 2013a

**McFadden A, Gias E, Stevens McFadden F, Pulford D.** Risks of outbreaks of anaemia associated with Theileria orientalis Ikeda in cattle. *Vetscript* 26 (12), 32-3, 2013b

**McFadden AMJ, Heath ACG, Fairley R, Trolove P, Pulford DJ.** Theileria (Ikeda) associated bovine anaemia in a West Coast dairy farm. *Vetscript* 28 (1), 2015