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The effect of month, farm type and latitude on the level of anaemia associated with *Theileria orientalis* Ikeda type infection in New Zealand cattle naturally infected at pasture

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ABSTRACT

Commencing in 2012, an epidemic of infectious bovine anaemia associated with *Theileria orientalis* Ikeda type has been present in New Zealand. The aims of this study were to analyse the temporal and spatial effects of *T. orientalis* Ikeda type infection on the sample submission rates and haematocrits of infected cattle over the first two years of the New Zealand epidemic. The data were collected from 30/08/2012 to 28/11/2014 and included all samples that met the case definition for *Theileria* associated bovine anaemia (TABA) and tested positive for *T. orientalis* Ikeda type by PCR. The sample submission rates by month and farm type were highly seasonal with dairy farm submissions peaking in September a month before beef farm submissions peaked. A second lesser peak of dairy farm submissions in April was absent for beef farms. A mixed effects model was fitted to the data and showed a significant interaction between farm production type (dairy or beef) and month of sampling ($p = 0.006$) and between latitude and month of sampling ($p = 0.024$). The estimated haematocrit, adjusted for month and latitude, for dairy cattle = 0.125 (95%CI 0.121–0.129) and for beef cattle = 0.151 (95% CI 0.138–0.165), $p < 0.0001$. This research shows that infected beef animals tend to be less severely affected than dairy animals and that the month of sampling and latitude of the sampled farm have significant and interacting effects on the level of anaemia associated with *T. orientalis* Ikeda type infection.

1. Introduction

Commencing in 2012, an epidemic of infectious bovine anaemia associated with *Theileria orientalis* Ikeda type has been present in New Zealand (McFadden et al., 2013). *T. orientalis* Ikeda type is one of eleven recognised allelic types of *T. orientalis* and is almost certainly the most pathogenic (Hammer et al., 2016). *T. orientalis* comprise tick-borne obligate intracellular apicomplexan haemoprotozoan parasites of cattle around the world (Aktas et al., 2006) and, except for the Ikeda type, are only rarely associated with severe disease outbreaks (Watts et al., 2016). To the best of our knowledge 4 of the 11 types of *T. orientalis* are presently found in New Zealand cattle, Type 1 (Chitose), Type 2 (Ikeda), Type 3 (Buffeli) and Type 5 (Pulford et al., 2016b). The Chitose and Buffeli types have probably been present in New Zealand since 1982, and have been sporadically associated with mild outbreaks of bovine anaemia in the upper North Island (James et al., 1984; Rawdon et al., 2006; McFadden et al., 2011).

The Ikeda type is associated with a pandemic of infectious bovine anaemia originating in East Asia before spreading first to Australia in 2006 (Kamau et al., 2011a; Eamens et al., 2013) and then New Zealand in 2012 (McFadden et al., 2013; Pulford et al., 2016a). The source of the Australian outbreak is not known, but historically the disease has long been described in Japan and Korea (Onuma et al., 1998), and the importation of infected Wagyu cattle from Japan via Guam is suggested as the likely incursion pathway (McFadden and Marchant, 2015). Similarly, the spread of disease to New Zealand probably followed the live importation of cattle from Australia prior to 2012. Little is known about the pathogenicity of Type 5 but it is presumed to be benign (Pulford et al., 2016a), it too was not found in New Zealand until 2012.

There is only one competent arthropod vector for *T. orientalis* in New Zealand, the ixodid tick *Haemaphysalis longicornis*, and its lifecycle and distribution have been thoroughly reviewed (Heath, 2016) and modelled (Lawrence et al., 2017c). Briefly, in New Zealand the *H. longicornis* life cycle is usually completed within 12 months, with overwintering nymphs mainly engorging from July to September, adults from November to December and larvae from February to April (Fig. 2,

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Heath, 2016). Once infected, cattle probably remain infected and infectious for life (Onuma et al., 1998) and although non-tick associated, mechanical spread of infection is also described through biting flies, sucking lice or hypodermic needles (Hadi and Al-Amery, 2012; Fujusaki et al., 1993; Hammer et al., 2015, 2016) it is not thought to be important in the epidemiology of *Theileria* associated bovine anaemia (TABA). However, its involvement in some New Zealand TABA outbreaks cannot be ruled out altogether. Likewise, vertical transmission of infection from dam to calf across the placenta is unlikely to be significant in the epidemiology of the disease (Lawrence et al., 2016b; Hammer et al., 2016; Swilks et al., 2017).

Infection rarely leads to overt signs of disease (Fairley, 1992; Vink et al., 2016) but when clinical disease is observed then clinical signs consistent with hypoxia and extravascular haemolysis are reported (McFadden et al., 2013; Lawrence et al., 2017a), with a case fatality rate of about 25% (Vink et al., 2016). The disease is highly seasonal with a major peak observed in October and a lesser one in April (McFadden et al., 2016b). Whereas the spring peak is easily explained by the feeding activity of over-wintered infected nymphs, the autumn peak does not correlate well with the active tick stage since it encompasses the period of larval questing and larvae are considered to hatch uninfected (Higuchi, 1985). Although the seasonality of the epidemic curve in New Zealand has been documented there has been no investigation of the effect of month, farm type, longitude or latitude on the level of anaemia observed in the individual diseased animals and the sample submission rates. The aim of this study was to test the hypothesis that the effect of *T. orientalis* Ikeda type infection on the haematocrit and the case submission rate is significantly different between beef and dairy farms, across different months of the year and across different latitudes and longitudes.

2. Materials and methods

2.1. Data collection

This was a retrospective observational study using data collected by the New Zealand Ministry for Primary Industries (MPI) in response to the epidemic of TABA. The data analysed in this study were collected from 30/08/2012 to 28/11/2014 and included the sampling date, the farm production type (whether dairy, beef or dry stock), the PCR test result, the haematocrit, the Cartesian X and Y coordinates (New Zealand Transverse Mercator 2000 projection) of the sampled farm and the number of piroplasm infected red blood cells (RBC) per 1000 RBC (when carried out). Data on animal age was rarely entered by private veterinarians on the laboratory submission forms so consequently was not routinely recorded in the *Theileria* data base. Farm production type was categorised as dairy, beef or dry based on the description in the AgriBase database maintained by AsureQuality Limited (www.asurequality.com). The spatial coordinates of the centroid of each farm were obtained from Farms on Line (http://farmsonline.maf.govt.nz/). All the farm data and laboratory results were entered and maintained in an Access 2007 data base (Microsoft Corporation, Redmond, Washington, U.S.). Initially MPI were responsible for keeping the *Theileria* data base but this was contracted out to AsureQuality (www.asurequality.com) at the end of 2013 and data recording officially finished at the end of November 2014.

2.2. Haematology testing

All the haematology work was completed at the private regional veterinary laboratories (New Zealand Veterinary Pathology (NZVP) and Gribbles Veterinary Pathology) who received blood samples from suspect cases of TABA, submitted by private veterinarians in the field. For each blood sample a thin blood smear was prepared, stained with Leishman's stain and examined under oil immersion using light microscopy. Erythrocytes were examined for the presence of signet or coma ring-shaped organisms consistent with *Theileria* piroplasms and the number of *Theileria* infected red blood cells per 1000 red blood cells observed were counted and used as a measure of infection intensity. This counting requirement proved to be too time consuming and was later simplified, by MPI, to just reporting if piroplasms were present on the blood smear. The haematocrit (HCT) was measured for each submitted sample using either the Sysmex XT-2000i (Sysmex Corporation, Japan) or the Cell-Dyn 3700 (Abbott Laboratories, Philippines) automated haematology analyser if the samples were sent to NZVP or Gribbles Veterinary Pathology respectively. The sampled animals were classed as anaemic if the haematocrit (HCT) $\leq$ 0.24 L/L (Riond et al., 2008).

2.3. Molecular testing

The molecular testing was completed either through the Animal Health Laboratory (AHL, Wallaceville, Wellington, New Zealand) or through the private regional veterinary laboratories. For a full description of the molecular techniques undertaken see Pulford et al. (2016b). Briefly, EDTA–treated blood samples were routinely diluted 1:5 in sterile water prior to DNA extraction, whereas serum samples were used undiluted. Samples were processed on a Corbett Extractorgene robot (Qiagen, Germany) to extract DNA which was eluted in 200 μL of diethylpyrocarbonate treated sterile water. All purified DNA samples were subject to an 18S quantitative PCR (qPCR) to measure sample consistency and the absence of PCR inhibitors. Samples were then analysed using an Ikeda type–specific TaqMan qPCR assay. The qPCR results with a quantification cycle threshold $\leq$ 38 were recorded as positive, > 38 as suspicious and samples with no signal were considered negative. PCR analysis was carried out solely by the AHL until January 2014 when the work was ceded to the private regional laboratories, where the same molecular technique developed at the AHL was used.

2.4. Data manipulation and statistical analysis

The analysis was restricted to only those samples that met the following requirements; the sampled bovine was anaemic (HCT $\leq$ 0.24 L/L), *Theileria* were identified on the blood smear by light microscopy, and the sample was positive by PCR for *T. orientalis* Ikeda type.

In the early part of 2013, MPI commissioned multiple samples from 26 recently infected cattle herds to gain a better understanding of the within herd prevalence and epidemiology of TABA (Lawrence et al., 2018). These data were removed since they were purposively sampled and would likely bias the results. Coincidentally there were several farms, not included in the prevalence study, where the consulting veterinarians independently submitted many samples. Although it was conceivable that these farms were experiencing an extensive TABA outbreak, it was also highly likely that the veterinarians' sampling protocols were influenced by the knowledge that MPI were fully subsidising the PCR costs. These multiple samples clearly represented an additional challenge to the analysis. On the one hand, it was considered important to reflect genuine increased seasonal or farm type susceptibility but on the other without the bias of excessive non-case based sampling. As a compromise, a random sub-sample of up to 3 Ikeda-positive anaemic submissions was made from all submissions that met the full case definition, for the same farm, on the same sample date. Each farm-sample date was then given a unique ID. If the same farm later submitted additional samples, which again met the case definition, a random sample of up to 3 submissions was again selected and tied to the later submission date with a new unique ID. Only beef or dairy farms and only North Island cases were included in the analysis; the dry stock cases and the two South Island cases were dropped.

A two-sample *t*-test was used to test whether there was any systematic bias in the estimated HCT between the two labs, given that the different laboratories used different haematology analysers. A plot of