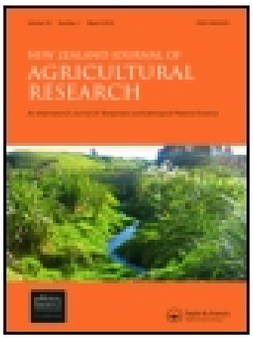

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Genetic susceptibility to Theileria orientalis (Ikeda) in Angus- and Hereford-sired yearling cattle born to dairy cattle on an endemically infected farm in New Zealand

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ABSTRACT

Theileria orientalis (Ikeda) was first detected in New Zealand in 2012, becoming endemic in most of the North Island, and can cause incidences of anaemia and death. Research has been performed in New Zealand on the incidence and severity of infection. Through this research anecdotal evidence has been found to suggest a potential genetic susceptibility component in the response of the host to the parasite. To investigate the genetic susceptibility of cattle to *T. orientalis* (Ikeda), 99 calves born in 2016 as part of a separate experiment and grown in six grazing herds, were examined for their response to the parasite. In addition to measuring live weight, two blood collections were taken in the first four months of life which were used to measure packed cell volume by haematocrit and qPCR to assess parasite load. Heritability was calculated and was low for parasite load, but the result indicates that there is some potential to increase resistance through selective breeding. The mechanisms of the differences in parasite load were not elucidated in this research; however, significant variation was found among herds and sires, indicating that both genetic selection and environmental management could be utilised to reduce parasite load in growing calves.

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Theileria orientalis (Ikeda); heritability; anaemia; susceptibility

Introduction

Theileria orientalis (Ikeda) is now endemic in much of the North Island of New Zealand, after being first detected in the country in 2012 (Pulford et al. 2016). Calves are born naïve to the disease and become infected once exposed to infected ticks (Lawrence et al. 2016). The timing of this infection varies with tick prevalence and season (Lawrence et al. 2018b), but previous work has shown high infection rates in spring-born beef calves by 4–6 weeks of age in regions with high tick prevalence (Lawrence et al. 2019b). This means that almost all calves born in infected regions are likely to encounter *T. orientalis* (Ikeda) and face a disease challenge as a result.

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The acute phase of infection can cause anaemia in cattle and a range of clinical signs may be seen alongside that anaemia including lethargy, reduced milk production, and inappetence (Watts et al. 2016; Lawrence et al. 2017). Experimental infection in bulls showed that infection is characterised by an increase in parasite load, followed by a decline in haematocrit around four weeks post-infection (Lawrence et al. 2018a). Approximately 16 weeks after infection, haematocrit stabilised to pre-infection level. Parasite load decreased from the initial peak, yet even 20 weeks after infection, bulls had detectable parasite loads (Lawrence et al. 2018a). The experimental infection of bulls was conducted on a property without a significant tick population, to prevent natural infection of the bulls during the experiment. In comparison, cattle on properties with a dense tick population are likely to experience ongoing reinfection and parasite challenge (Lawrence et al. 2019a) and will need to continually suppress this challenge to remain productive.

The incidence and severity of infection was well documented during the initial outbreak of the disease in New Zealand, among different herds, with the inter-quartile range 0.36–2.07% for incidence risk and 0.00–0.66% for cumulative mortality (Vink et al. 2016). The reasons for differences among herds are not clear; however, variation in underlying nutritional stress, presence of other concurrent disease challenges and physiological state are all likely factors. In addition, anecdotal evidence from some farmers that experienced more severe outbreaks indicated a suspected genetic component, with particular lines of cattle more affected than others.

A genetic component to susceptibility may also explain some of the variation observed among and within herds. This hypothesis would not seem unreasonable given that some beef and indigenous breeds appear to have a lower susceptibility to *T. orientalis* infection compared to some dairy breeds (Terada et al. 1995; Higuchi et al. 2003; Sivakumar et al. 2017). An explanation for this was suggested by the finding that Wagyu cattle have significantly lower levels of I-active ganglioside in their erythrocytes compared to Holstein-Friesian cattle (Watarai et al. 1995). I-active ganglioside is important for the attachment and invasion of erythrocytes by *T. orientalis* merozoites (Watarai et al. 1994).

Some farms in endemic stable areas continue to experience ongoing losses in their herds each year, and others in endemic unstable areas only experience high losses when their naïve cattle are transiently exposed to infected ticks. There are currently no known preventative treatments for *T. orientalis* (Ikeda) infection. Some therapeutic, treatments, such as blood transfusions and the drug buparvaquone, have proved useful in dairy cattle, however, their usefulness is limited in beef cattle due to the logistical difficulties of administering blood transfusions and the prolonged meat withholding period legally required for buparvaquone (Lawrence et al. 2021). Therefore, there is an urgent requirement to explore the potential for a genetic role in *T. orientalis* (Ikeda) susceptibility, particularly for the beef sector of New Zealand.

Measuring variation in the extent of *T. orientalis* (Ikeda) infection requires definition of an appropriate phenotype. An ideal phenotype would be the parasite load and the anaemia response to acute infection; however, this phenotype may be heavily influenced by the timing of infection in relation to measurement, the extent of the infective dose, and the physiological state of the animal at the time of infection, all of which cannot be controlled or effectively measured under commercial conditions on an infected property. An alternative, more practical phenotype could be the parasite load during the

chronic phase of infection, when all cattle have had a prolonged period to adjust to the infection. Furthermore, such a measurement should be conducted at a time of year when ticks are at a less active phase of their seasonally driven life cycle to minimise the influence of the current disease challenge on the measurements.

The aim of the present experiment was to assess the variation in parasite load in late autumn in 10-month-old calves that had likely been exposed to *T. orientalis* (Ikeda) within the first four months of life, to determine the heritability of parasite load, and to assess the relationship between parasite load, haematocrit level and live weight of the calves.

Materials and methods

Animals and management

This experiment used 449 calves born in July–September 2016 as part of a progeny test comparing performance of Hereford and Angus bulls used over Friesian and Jersey-Friesian crossbred dairy cows in New Zealand, which were a subset of those reported in Martín et al. (2020). Forty-eight bulls had been randomly assigned for mating via artificial insemination with the mixed-aged cow herd at Limestone Downs dairy farm in Port Waikato, New Zealand. All calves had parentage assigned at birth using commercial DNA-parentage technology (Zoetis, Dunedin).

Calves were collected from their dams daily, within 24 h of birth, and reared in a calf shed, either on the Limestone Downs dairy farm ($n = 335$), or at a commercial calf rearing facility in Tirau ($n = 114$). Calves reared at Limestone Downs grazed on the dairy platform once they were released from the calf shed at approximately four weeks of age, before moving to the neighbouring Limestone Downs sheep and beef farm at 100 kg live weight, around 14 weeks of age. Calves reared in Tirau grazed outside from around 5–6 weeks of age and returned directly to Limestone Downs sheep and beef farm at 100 kg, around 14 weeks of age. Only early-born calves were reared in Tirau, returning in October and early November 2016.

In December 2016, once all calves had been weaned and moved to the sheep and beef farm, calves were allocated to one of six grazing herds, on the basis of sex (three herds of steers, and three of heifers) and live weight within sire group, so that each sire had calves in every herd wherever possible and the heaviest, middle and lightest third of steers from each sire were in the big, medium and small steer herds, respectively, and likewise for heifers. Calves remained in these herds grazing hill country pasture on the sheep and beef farm for the duration of the experiment.

Measurements

Two subsets of calves that were reared at Limestone Downs were blood sampled on 2nd November ($n = 50$) and 7th December 2016 ($n = 49$) to ascertain the percentage of calves that were infected at that time. Calves in the second group were younger than those in the first group, and both groups had been at pasture for eight weeks prior to sampling.

Heifer calves were weighed on 22nd May 2017 and steer calves on 16th June 2017. A blood sample was collected on 28th or 29th May 2017, when the calves were

approximately ten months old. Blood samples were collected from the tail vein into 10 ml EDTA vacutainers and immediately stored on ice, before being refrigerated for 16–36 h.

Duplicate capillary tubes were filled from each blood sample and measured for haematocrit before samples were frozen at −20°C. A data entry error meant that some ($n = 85$) haematocrit levels were not recorded.

DNA extraction

DNA was extracted from 50 µL of the collected EDTA blood using the NucleoMag® Vet kit (Machery-Magel, Düren, Germany) processed on a KingFisher Flex System (ThermoFisher, Massachusetts, USA). Extracted DNA was quantified utilising the Qubit™dsDNA HS Assay Kit and Qubit 2.0 Fluorometer (ThermoFisher, Massachusetts, USA). Extracted DNA was stored at −20°C until analysis. Negative controls were produced by extracting DNA from cattle blood samples that were known to be naïve to *T. orientalis* (Ikeda).

Quantitative PCR

Quantitative PCR (qPCR) on the extracted DNA was performed using the primers and probe previously described for *T. orientalis* (Ikeda) by Pulford et al. (2016) All primers and probes were sourced from Integrated DNA Technologies (Iowa, USA). qPCR was performed with the following conditions: 1 x PerfeCTa qPCR ToughMix (Quantabio, Massachusetts, USA), 0.5 µM of each primer, 0.4 µM of the probe and 5 µL of template DNA in a final volume of 20 µL. Thermal cycling was performed in a Rotor-Gene Q (Qiagen, Düsseldorf, Germany). Thermal cycling conditions were: 95°C for 2 min, followed by 45 cycles of 95°C for 15 s and 60°C for 30 s, with fluorescence capture on the 60°C step. Standard curves were produced utilising a serial dilution of a plasmid from a previously produced clone of a section of the *T. orientalis* Ikeda MPSP gene (provided by K. Gedye), and ranged from 100 pg to 10 fg/µL of the plasmid. Runs were analysed using the Rotor-Gene Q software and exported for further statistical analysis. The standards were also the positive controls, with negative controls being PCR grade water and the DNA extracted from naïve cattle. The average of two qPCR measurements for each sample was used for the analysis.

Statistical methods

Descriptive statistics were obtained with the MEANS procedure of SAS v9.4 (SAS Institute, Carey, NC). Three sires with fewer than five progeny present at sampling were excluded from the analysis.

Analysis of variance for the dependent variables (haematocrit, parasite load and live weight) were performed with the MIXED procedure of SAS v9.4 (SAS Institute, Carey, NC). The mixed model included the fixed effects of rearing location, grazing herd, breed of sire, sire within breed, birth of date as covariate and random residual errors. The residual errors were assumed with mean zero and variance σ_e^2. To examine the relationship between variables, parasite load and haematocrit were added separately as covariates to models for live weight. Similarly, parasite load was added to the model for haematocrit. Least squares means of the dependent variables for each class of the fixed effects were obtained and used for mean multiple comparisons using Fisher's least significant test.

Variance components for estimation of heritability (h^2) were obtained using the ASReml 3.0 software package (Gilmour et al. 2015) with the same del described above

but considering sire within breed as random effect with expected mean value zero and σ_s^2. The h^2 for each dependent variable was calculated as $h^2 = \sigma_a^2 /(\sigma_s^2 + \sigma_e^2)$., where σ_a^2 is the additive genetic variance calculated as $\sigma_a^2 = 4 \times \sigma_s^2$.

Results

Forty-four percent of calves tested on 2nd November were infected with *T. orientalis* (Ikeda), and this increased to 94% of calves tested on 7th December, indicating that all the calves in this experiment were likely to have been infected several months prior to the May sampling. All calves were infected at the May sampling. Anaemia (haematocrit < 25%) was detected in 11% of calves sampled in November or December, all of which were positive for *T. orientalis*, whereas only one calf (0.2%) was anaemic in May, with a haematocrit of 24%. That calf was born in late July and reared at Limestone Downs so was unlikely to be experiencing an acute infection, but it had not been included in the subset of calves tested earlier.

Residuals for both haematocrit and live weight were normally distributed, while parasite load was right-skewed with a small number of high values. Residuals were normally distributed after log transformation of parasite load. Mean live weight was 230 kg, with a large standard deviation, which was reflected in the 173 kg range and 13% CV. Haematocrit had a mean of 31.3%, and a smaller 8% CV Table 1.

Neither live weight, haematocrit or parasite load were affected by the breed of the sire, but both live weight and parasite load varied with grazing herd (Table 2). Sire of calf contributed to variation in live weight and log-transformed parasite load, but not haematocrit (Table 3; Figures 1 and 2).

Live weight was not influenced by haematocrit ($P = 0.938$) or parasite load ($P = 0.727$), and haematocrit was not affected by parasite load ($P = 0.621$; data not shown).

Discussion

Heritability of parasite load was moderate at 25% and indicated some potential to increase resistance to *T. orientalis* (Ikeda) through genetic selection for reduced parasite load. There was no phenotypic relationship between parasite load and live weight in this experiment, so further investigation into whether parasite load is the most appropriate trait to measure is needed. Perhaps the desired phenotypes are animals that are growing well despite a high parasite load, or simply animals that are growing well irrespective of parasite load. Selection of animals that grow well irrespective of parasite load is already occurring in all endemically infected nucleus herds and would require no change to existing methods of selection.

The mechanisms underlying the differences in parasite load could not be determined from this experiment. There is a known genetic basis to tick count in cattle, with

Table 1. Descriptive statistics for live weight (kg), haematocrit (%), and parasite load (pg/ml) of 479 10-month-old calves on an endemically infected farm in New Zealand, measured in May.

	n	Mean	S.D.	Median	Range
Live weight (kg)	449	230	29	231	148–321
Haematocrit (%)	364	31.3	2.4	31.3	24.0–40.5
Parasite load (pg/ml)	449	1.63	1.70	1.18	0.02–13.27

Table 2. Live weight, haematocrit and parasite load measured in May for 10-month-old Angus-sired and Hereford-sired calves grazed in six different herds on an endemically infected farm in New Zealand.

		Live weight (kg)	Haematocrit (%)	Parasite load[1] (pg/ml)
Breed				
	Angus-sired	229 (226–232)	31.3 (30.9–31.7)	0.92 (0.80–1.06)
	Hereford-sired	230 (227–233)	31.4 (31.0–31.8)	0.84 (0.73–0.96)
	P value	0.781	0.783	0.363
Grazing herd				
	Steers A	258[a] (254–262)	31.2 (30.6–31.8)	2.25[a] (1.83–2.76)
	Steers B	241[b] (238–245)	31.9 (31.3–32.4)	1.74[a] (1.43–2.12)
	Steers C	234[c] (229–238)	31.6 (30.9–32.4)	0.93[b] (0.74–1.16)
	Heifers A	221[d] (216–225)	31.0 (30.3–31.7)	0.88[b] (0.70–1.11)
	Heifers B	224[d] (220–229)	31.0 (30.3–31.7)	0.81[b] (0.65–1.00)
	Heifers C	200[e] (195–204)	31.6 (30.9–32.3)	0.18[c] (0.14–0.22)
	P value	<0.001	0.333	<0.001

Note: Values are means and 95% confidence intervals.
[a,b,c,d,e]Values within effects without superscripts in common differ at $P < 0.05$.
[1]Values for parasite load are back-transformed

heritability estimates in Australian cattle ranging from 13% to 44% (McKinnon et al. 1991; Henshall 2004). This means it is possible that the differences in parasite load among progeny groups observed in this study may be partially or fully explained by differences in parasite exposure due to variation in tick load. The heritability estimate for parasite load in this experiment was in the range of other parasite traits, which ranged from 6% for buffalo fly burden, to 28% for worms and 34% for ticks (McKinnon et al. 1991), and also consistent with reports of low to moderate heritability for a variety of disease traits including bovine keratoconjunctivitis, mastitis, foot-and-mouth disease and paratuberculosis (Morris 2007).

Progeny from a single Angus sire had a markedly greater parasite load than other progeny groups. Genome-wide association studies would be worthwhile to identify loci of relevance for parasite load. Significant quantitative trait loci have been identified for resistance to *Rhipicephalus (Boophilus) microplus* (Otto et al. 2018), so similar genetics for resistance to *Haemaphysalis longicornus* (the tick of relevance in New Zealand) could be performed. Potentially, this outlier sire has a significant allele for this trait: however, it could also be a spurious finding as the sire had only six progeny.

No clinical cases of theileriosis were reported for these animals. However, calves were farmed under extensive grazing conditions on a large property, so they were often in paddocks of around 60 ha in steep terrain with many places where an anaemic calf could rest undetected. Nine calves (2%) went missing, presumed dead, between December and May, which is consistent with industry norms for cattle of this age farmed under these conditions, indicating that significant losses from *T. orientalis* did not occur in this

Table 3. Estimates (±sem) of sire (σ_s^2), residual (σ_e^2), additive genetic (σ_a^2) and phenotypic (σ_p^2) variances and narrow-sense heritability (h^2) estimates for live weight, haematocrit and transformed parasite load, for 10-month-old calves on an endemically infected farm in New Zealand.

	σ_s^2	σ_a^2	σ_e^2	σ_p^2	h^2
Live weight (kg)	25 (±12)	100 (±48)	267 (±19)	292 (±21)	0.34 (±0.15)
Haematocrit (%)	0.0	0.0	5.83 (±0.44)	5.83 (±0.44)	0.00 (±0.13)
Log(parasite load) (log pg/ml)	0.03 (±0.02)	0.13 (±0.07)	0.49 (±0.03)	0.52 (±0.04)	0.25 (±0.14)

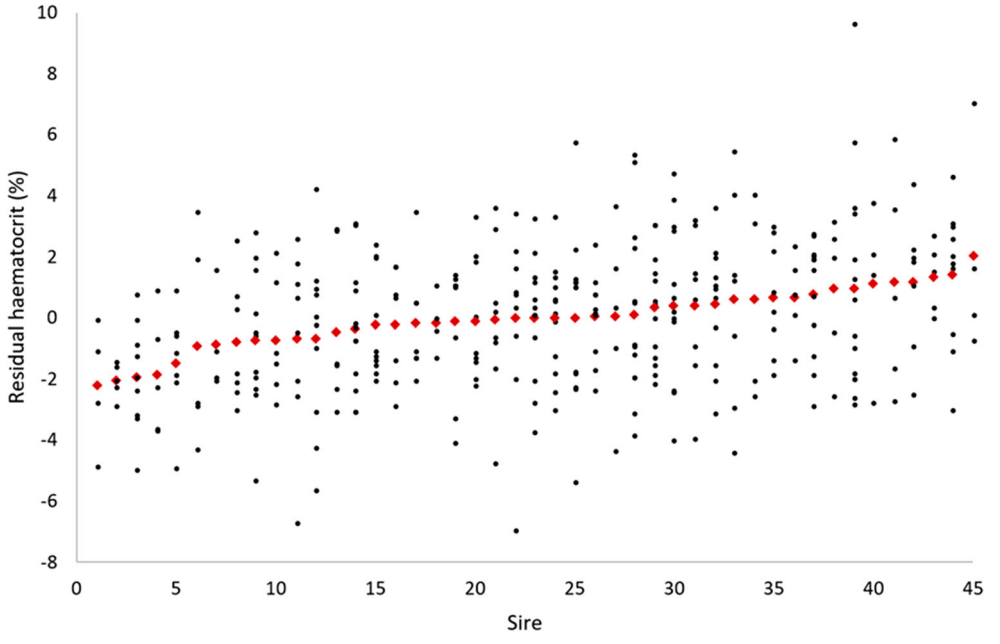

Figure 1. Mean residual haematocrit for each sire group (red symbols) and residuals of individuals within each sire group (black symbols) for progeny of Angus and Hereford bulls at ten months of age. Sires are ordered from least to greatest mean for this trait.

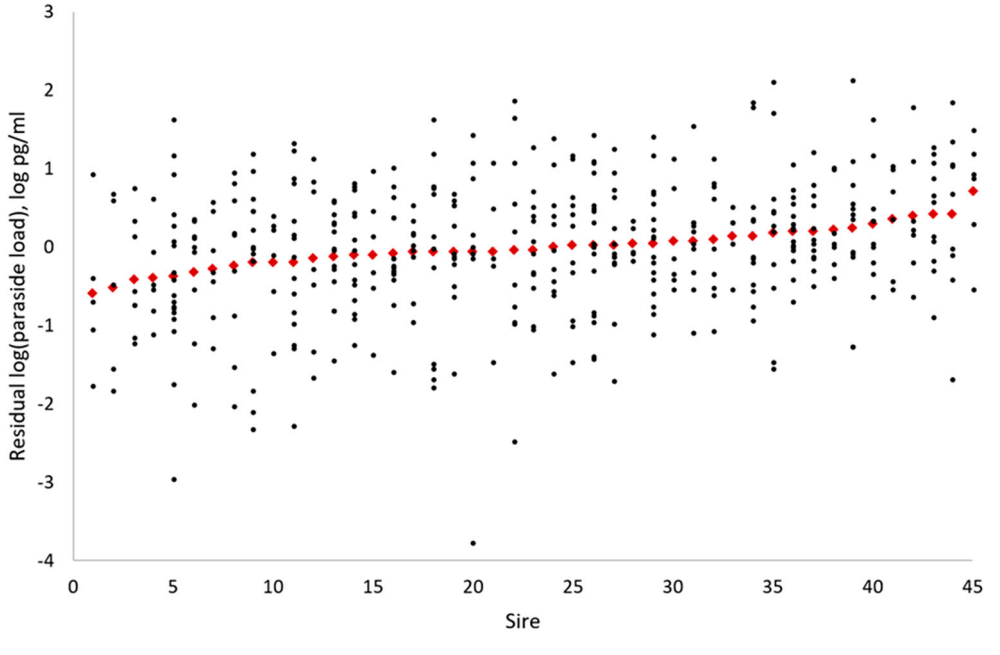

Figure 2. Mean residual log-transformed parasite load for each sire group (red symbols) and residuals of individuals within each sire group (black symbols) for progeny of Angus and Hereford bulls at ten months of age. Sires are ordered from least to greatest mean for this trait.

herd. The parasite load reported here is lower than the average parasite load reported for dairy cows over the whole lactation on the same property, of 4500 theileria organisms per μl, but two of the grazing herds were comparable to the April value for the dairy herd of around 1800 theileria organisms per ml (Lawrence et al. 2019a). These results indicate that the testing had been successfully timed to coincide with a period of lower tick prevalence on the farm. However, the variability among grazing herds highlights that there were still considerable differences in the environments of the different herds that presumably resulted in differences in tick prevalence. The farm on which the cattle were grazed spans some 2500 ha, and includes a variety of topography, herbage cover and climatic conditions. Information on grazing area was not recorded for individual herds but the herds were distributed throughout the farm, so it is likely that differences in environmental conditions contributed to differences in tick prevalence in the different paddocks.

Significant variation in parasite load was observed among herds and among sires, indicating that both environmental management and genetic selection could be used to manage *T. orientalis* parasite load in growing calves. In spite of this, no relationships were observed between *T. orientalis* parasite load, and haematocrit or live weight during the chronic phase of the infection, so further research is required to determine the value of such approaches.

Disclosure statement

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