A Longitudinal Study of Parasitosis With Genotypes of *Theileria Orientalis* in Calves and Introduced Cattle at Dorrigo, New South Wales, and the Effect on Weight Gains.

David Emery (✉ david.emery@sydney.edu.au)

University of Sydney <https://orcid.org/0000-0003-2722-6854>

Shaojing Wang

University of Sydney

Chantelle Loo

sydney University

Chris Shirley

Dorrigo Vet Clinic

Research

Keywords: Theileria orientalis, ikeda, parasitosis, weight gain, cattle.

Posted Date: October 23rd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-93408/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: In regions with endemic *Theileria orientalis*, recovered cattle are carriers, leaving newborn calves and introduced stock as the most susceptible groups to develop clinical disease after tick infestation.

Methods: To gain information on the kinetics of infestation and development and effects of parasitosis by virulent Theilerial genotypes ikeda and chitose and the “benign” genotype, buffeli, this study sampled a total of 150 calves from 3 farms in Dorrigo, Australia, a region with multiple genotypes of the parasite. In addition, 30 introduced beef weaners were bled and weighed for 6 months after arrival.

Results: In both cohorts, parasitosis with the *T.orientalis* ikeda and chitose (genotypes 1 & 2) occurred within 3-4 weeks in >95% of the groups, with maximal gene copies generated around 5-7 weeks after birth or introduction, before declining. In contrast, parasitosis with *T.orientalis* buffeli (genotype 3), increased slowly, with around 80% of the population testing positive by PCR after 4 months. The parasitosis with the buffeli genotype did not exceed 40000 gene copies /ul and was similar to those exhibited by ikeda and chitose genotypes following the “first wave of parasitaemia” as cattle entered the carrier state. In the 30 introduced weaners, the early parasitosis with *T.orientalis* ikeda and chitose also caused significant reductions in haematocrit and incurred around 20Kg loss in weight gain; the latter had not been recovered by 6 months after introduction.

Conclusions: The results support previous findings in other endemic regions of Theileriosis, but imply that amelioration of the early high levels of parasitosis by the virulent genotypes could reduce the initial impact of Theileriosis on production, which appears much less affected once animals enter the carrier state.

1. Introduction

Endemic in Japan and Korea, the intracellular protozoal parasite *Theileria orientalis* has rapidly spread across South-eastern Australia, substantially impacting local cattle industries since 2006. Currently, 11 genotypes of *T. orientalis* (type 1 or Chitose, type 2 or Ikeda, type 3 or buffeli, types 4–8, and N1-N3) had been identified based on major piroplasm surface protein (MPSP) gene sequences (Sivakumar et al. 2014; Bogema et al., 2015). Of these genotypes, 1 and 2 cause the majority of clinical disease in cattle.

Early studies indicated that *T. orientalis* prevalence was high in New South Wales (NSW, 23.7%), Queensland (Qld., 56.8%) and Victoria (Vic.), 34.0%, with variability among regions of each state. Genotype chitose was the most common and widespread type (19.1–43.7% per state), with buffeli present in all states at a lower prevalence (10.8–24.8% per state) when studies were performed by Eamens (2012) and Eamens et al. (2013b), similar to results reported by Kamau et al. (2011). Since its early detection, this parasite has spread through Victoria and across to Western Australia (WA) and east into New Zealand (NZ; Hammer et al., 2015; McFadden et al., 2011).

For theilerial transmission and pathogenesis, sporozoites of *T. orientalis* inoculated from tick salivary glands during feeding attach and invade host leucocytes and following division, become schizonts. These can be seen transiently in lymph nodes, spleen, and liver with immunohistological staining around 10 days after infestation, but are not responsible for the pathology associated with the infection, unlike other species of *Theileria* such as *T. parva* (Kawazu et al., 1991). Maturation of schizonts results in uninucleate merozoites which parasitise red blood cells from around 8–10 days post inoculation. The ensuing rounds of asexual reproduction are responsible for the febrile episodes and the clinical manifestations of *T. orientalis*, including the signs associated with anaemia (pale mucous membranes, icterus, tachycardia, tachypnoea, weakness; Izzo et al., 2011; Hammer et al., 2016; McFadden et al., 2011). Parasitosis in red cells was detected in blood smears 12–16 days after tick application for *T. orientalis buffeli* (Stewart et al., 1987a), and approximately 20 days after ticks were seen for *T. orientalis ikeda* (Izzo et al., 2011), with variations in timing attributed to the quantum of infection. Consequently, clinical disease may not occur until around 4–6 weeks after infection, well after ticks had fed and gone. However, the timing is consistent with reports describing the introduction of susceptible stock into endemic areas (Eamens et al., 2013) and observations with calves (Hammer et al., 2015; Swilks et al., 2017).

In endemic zones, recovered cattle remain carriers, so that naive animals such as introduced cattle and newborn calves are most at risk from Theilerial infestation in coastal New South Wales (NSW), with initial parasitoses evident by PCR after 2–3 weeks (Swilks et al., 2017, Jenkins et al., 2015). Interestingly, several studies have indicated that the carrier state arising in recovered dairy cattle did not compromise subsequent productivity (Perrera et al., 2014; Lawrence et al., 2019a).

This study extended these findings in the mountain regions of NSW at Dorrigo, and included a longitudinal study over 6 months to determine the impact of Theileriosis on weight gains and examine correlations with packed cell volume (haematocrit; PCV) and level of parasitosis with the Theilerial genotypes (*ikeda*, *chitose* and *buffeli*).

2. Materials And Methods

The trials in this study conformed with requirements for animal health and well-being under the University of Sydney Animal Ethics committee (AEC) permit 2018/1328.

2.1 Detection of Theilerial genotypes in ticks and cattle from Stroud and Dorrigo

In a preliminary investigation, 10 ticks were collected from cattle on a beef property at Stroud (32.42S; 151.97E) and 30 ticks from each of 4 farms at Dorrigo (30.34S; 152.71E). The ticks were confirmed as *H. longicornis*. They were held in 100% ethanol before being cut longitudinally within a week of collection. The half with mouthparts was kept while DNA was extracted from the other segment for analysis.

A further resident 30 beef cattle from the same farm were bled to determine the level of endemicity of Theilerial genotypes. Around 5 ml blood was into EDTA vacuum tubes (Vacurette, Griener Bio-one). Each tick and blood sample was subsequently tested for infestation with Theilerial genotypes by PCR using the Ausdiagnostics® kit.

2.2 Samples for detection of Theilerial genotypes in calves and introduced cattle at Dorriggo

Blood samples were collected into EDTA-vacuum tubes from 111 calves from the Spring calvings on 3 local properties were bled around 8 weeks after the start of calving in October 2017 and 54 calves on 2 of the farms were sampled again in October 2019. Since calvings occurred over 6–8 week periods, the ages of calves ranged from 4–10 weeks when blood samples were collected. On 2 of the farms, a record of birth dates enabled age-related comparisons with theilerial parasitosis in both years. In a second study, 30 beef weaners from a non-endemic region were followed by bleeding and weight measures every 3 weeks for 6 months after introduction to a Dorriggo farm in late February 2020. These were used to compare the onset of Theileriosis and its impact on productivity and for comparison with the age-prevalence data from the calves in local properties above.

Blood samples from both studies were analysed for Theilerial genotypes using the Ausdiagnostics® kit and the PCV was measured. RFID tags on the introduced cattle enabled weight measures at each bleeding time and the Average Daily Liveweight Gain (ADLG) was then calculated over each 3-weekly interval. The actual mean liveweights were also compared against expected regional benchmarks of 0.8 kg/day and both measures were used to determine the impact of Theileriosis on weight gains.

2.3 Sample analyses

For all diagnostic PCR, DNA extraction from 200 ul of blood sample was performed using the KingFisher® MO BIO PowerMag® Microbiome robotic program according to the manufacturer's protocol for blood. The KingFisher PowerMag Blood DNA/RNA Isolation protocol program on the KingFisher Duo™ platform robot completed the DNA isolation. The final eluted volume ready for qPCR was 100 ul.

DNA was extracted from ticks using the recommended protocol for insects in the MagMAX CORE Nucleic Acid Purification Kit (ThermoFisher).

Theilerial PCR runs were conducted with 11 eluted DNA samples, one indicator sample, MasterMixes (Theileria), oil, water, and step 2 strips loaded separately into the AusDiagnostics® Easy-Plex™ Processor, which provided sequences for Theilerial genotypes: Ikeda, Buffeli, Chitose, and type 5. Both automatic assay set-up and Step 1 PCR were accomplished in this machine. Samples were then transferred into the Bio-rad CFX96 analyser to perform the Step 2 PCR. Real-time PCR Ct-values were collected for data analysis. Relative gene copy numbers per ul of blood (GC/ul) were calculated for both pan-Theileria and Theilerial genotypes from spiked standards in the assay. Tick samples were simply recorded as positive or negative at a cycle take-off (Ct) where positivity was < 30.

2.3 Statistical analysis

Statistical analysis was performed using GenStat™ software. Raw data were log_e transformed and five linear mixed models for PCV, Ikeda, Chitose and Buffeli gene copies per µL and Ct values were developed for each dataset. The fixed effects were Day, Property and the interaction between Day and Property for calves. The random effect was Animal ID. P-values were calculated for each fixed effect in each model to determine whether they were significant. For significant fixed effects, the differences in the predicted means for each factor level were compared to the Least Significant Differences (LSDs) at significance level 0.05 to determine whether pairwise comparisons were significant. If the interaction fixed effect was significant, no further pairwise comparisons were determined for the other fixed effects.

Predicted means for PCV, Ct values and GC/µL for Theilerial genotypes within treatment groups were presented with standard error (SE) bars.

3. Results

3.1 Parasitosis in ticks from Stroud and ticks and cattle from Dorrigo

All ticks were identified as *H.longicornis*. More than 80% of ticks from Stroud and Dorrigo farms as well as all of the 30 Dorrigo cattle tested positive for multiple Theilerial genotypes (ikeda, chitose, buffeli and type5), indicating a very high level of Theilerial endemicity in both regions (Table 1).

Table 1

The presence of parasitosis with 4 genotypes of *T.orientalis* in ticks and cattle from Stroud and Dorrigo

Samples and Location			Number positive for <i>T.orientalis</i> genotype			
Host	Location	Number tested	Chitose (type 1)	Ikeda (type 2)	Buffeli (type3)	Type 5
Ticks	Stroud	10	10	8	10	10
Ticks	Dorrigo	120	112	105	115	102
Cattle	Dorrigo	30	30	30	29	13
Results are expressed as the number positive by PCR in the tested population						

3.2 Age-related levels of *T.orientalis* genotypes in calves on Dorrigo farms

Parasitosis in 57 calves born in 2017 and 54 born in 2019 on the properties at Dorrigo are shown in Figs. 1 & 2. All calves were infested with *T. orientalis* ikeda with the youngest at 5 weeks showing the

highest levels of parasitosis as determined by PCR and expressed as Theilerial gene copies/ul blood (GC/ul; Fig. 1). The mean peak GCs/ul for *T.orientalis* chitose and ikeda were 2846 and 5413, respectively in 2017 and 953 and 807, respectively in 2019. As calves aged, there was a trend for lower levels of parasitosis with GC/ul for genotypes chitose and ikeda declining significantly by 3 months of age (Figs. 1 & 2, top), manifesting as a negative association between GC/uL and age over time (Table 2). In contrast, the levels of parasitosis with *T. orientalis* buffeli increased with age and the proportion of calves infected also increased to around 80% in animals by 4 months of age (Fig. 2, bottom). However, the GC/ul of *T. orientalis* buffeli reached a mean GC/ul of 1066 and 581 in 2017 and 2019, respectively, around 2–5-fold lower than those generated by *T. orientalis* ikeda (Figs. 1 & 2). Year had a significant effect on all three genotypes of *T.orientalis* (chitose $p < 0.001$; ikeda $p < 0.001$; buffeli $p = 0.57$). The interaction between age and year was only significant for *T.orientalis* ikeda ($p < 0.001$). The lines of best fit and correlation coefficients for each of the 6 comparative age-related parasitoses are shown in Table 2. There were no significant correlations between age and GC/ul for any of the 3 Theilerial genotypes.

Table 2
Equations for lines of best fit in Fig. 2, describing correlations between age and parasitosis with Theilerial genotypes in Dorrigo calves

Year	Theilerial genotype	Line of best fit (Y=)	Correlation (R ²)
2017	Ikeda	176.12x + 7477.4	0.0174
	Chitose	107.34x + 4104.3	0.0186
	buffeli	202.69x - 1309	0.2541
2019	Ikeda	-176.12x + 7477.4	0.0471
	Chitose	-107.64x + 2671.6	0.135
	buffeli	20.398x + 255.54	0.0078

3.3 Longitudinal changes in parasitosis with genotypes of *T.orientalis* in introduced cattle

The levels of Theilerial parasitism and average daily liveweight gain (ADLG) in 30 introduced weaners is depicted in Fig. 3. Animals became infected with *T. orientalis* within 3 weeks after arrival as detected by PCR. The parasitosis for *T. orientalis* ikeda and chitose was maximal at 6-weeks after arrival. Here the parasitosis peaked around means of 80000–200000 GC/ul blood for both genotypes. Subsequently, the parasitosis declined sharply over the ensuing 2 months to stabilise under 20000 GC/ul blood (Fig.). In contrast, the prevalence of *T. orientalis* buffeli increased more slowly, with infections in around 70% of the cattle by 3 months after arrival and in the whole 30 cohort by 6 months after arrival (Fig. 3). Cattle also developed a lower level of parasitosis ranging from 15000–40000 mean GC/ul blood throughout the monitoring period. By 12 weeks after arrival, level of parasitosis of all 3 Theilerial genotypes was similar (Fig. 3), indicating that the levels of parasitosis exhibited by the virulent genotypes after the first wave of parasitism were similar to those generated by *T.orientalis* buffeli throughout the monitoring period.

A sharp decline in ADLG and a parallel but later decline in blood packed red cell volume (PCV) was evident following and through the initial parasitaemia, particularly around the 2–3 months after arrival (Figs. 3 & 4). Once the initial wave of parasitosis passed after 2–3 months, both PCV and ALDG improved towards regional benchmarks of around 32 and 0.8 kg/day, respectively, in May (Figs. 3 & 4). ADLG declined again over the winter months while the parasitosis remained relatively stable (Fig. 3). PCVs recovered in the majority of the cattle by 4 months and for the remainder of the observation period towards mid-August, 6 months after arrival (Fig. 4).

3.4 Longitudinal changes in weight gains in cattle introduced to Dorrig

The mean weight at arrival in Dorrig was 155 kg. Based on an expected ADLG of 0.8 kg/day, the mean expected weight after 90 days would be 227Kg (Fig. 4). Since the mean observed weight at this time was 205Kg, the Theileria infestation cost around 22 kg over this period. As the PCV rose, the second reduction in ADLG from June to August was likely due to declining nutrition. The expected liveweight gain over these 3 months was 45 kg, some 25Kg short of expected gains at 0.8 kg/day (Fig. 4).

4. Discussion

H. longicornis has been confirmed as a biological vector (final host) for *Theileria orientalis* ikeda (Marendy et al., 2019). The tick occurs in the coastal areas of Victoria and New South Wales and extends northwards as far as Gympie in Queensland but is absent from large areas of Northern Australia where *Theileria* sp (*T.orientalis* buffeli) is present (Reik, 1982). *H.longicornis* was found as the principal tick species at Dorrig and Stroud. The majority of ticks at both locations contained DNA from multiple genotypes of *T.orientalis*, consistent with the presence of the same range and prevalence in bovine blood from Dorrig. In these 2 locations, *H.longicornis* consistently ingest bovine blood from cattle multiply-infected with several Theileria genotypes and subsequently appear able to re-infect naïve cattle with each of these genotypes, including *T.orientalis* buffeli. These data on vector competence, including our own results (Emery et al., submitted buffeli or MLA report) do not explain how *T.orientalis* buffeli is vectored around Dorrig and Stroud in NSW, as previous vector studies indicated that *H.bancrofti* and *H.humerosa* were likely vectors for *T.orientalis* buffeli in Queensland (Stewart et al., 1987a,b). However, as noted with the kinetics and age-related infections in naïve animals at Dorrig, *T.orientalis* buffeli infestations are the slowest to develop. Considering vector competence, this could arise for several reasons:

- since gene copies of *T.orientalis* buffeli are generally lower in blood than other Theileria genotypes, less parasites are ingested by the tick;
- *T.orientalis* buffeli may be selectively outcompeted for maturation in the tick (so less sporozoites inoculated); or,
- replicate more slowly in the host compared with the virulent genotypes; or,

- simply reflect that fact that *H.longicornis* does not transmit *T.orientalis* buffeli as readily or effectively as the *T.orientalis* ikeda and chitose genotypes.

In both introduced naïve cattle and calves born into endemic regions of Theileriosis, infection occurs rapidly for *T. orientalis* ikeda and chitose, and much more slowly with *T. orientalis* buffeli. From PCR measures, GC/ul of Theileria below 15,000 are considered to indicate mild infection, 15,000-300,000 indicates moderate infection, while clinically significant parasitosis is considered when GC/ul blood exceed 300,000 (Bogema et al., 2015). In the current study, 3 animals died in the wider introduced cohort, and clinical signs of anaemia were readily apparent in 4 of the monitored 30 animals around 6 weeks after introduction. Around this period after arrival, these affected animals had parasitoses of 120000–360000 and 55000–150000 GC/ul blood for *T.orientalis* chitose and ikeda genotypes, respectively. The differences between GC/ul levels in the two studies are likely to reside in the different diagnostic kits used, but relatively approximate one another.

In 30 calves sampled from the Dorrigo farms in 2017 and 2019 where birth dates were available, results confirmed that calves were readily and heavily infected within 4–5 weeks of birth, consistent with results from Gloucester, NSW (Swilks et al., 2017). Parasitosis was lower in 2019 than seen in 2017, likely due to earlier calvings in 2019 in attempts to avoid the height of the tick season in Spring. Similar to the results in the calves, beef weaners introduced to Dorrigo in late February 2020 were PCR-positive for Theilerial genotypes within 3 weeks after introduction and exhibited clinical Theileriosis within 5–6 weeks after arrival. This was unexpected as the seasonal life cycle of *H. longicornis* would suggest that larval ticks would be present in late Summer and would need to feed on infected cattle to transfer the infestation as nymphs later in the year. However, not only did these cattle arrive at Dorrigo late February after a monthly rainfall of 580 mm, but the outcome confirms that clinical Theileriosis can occur throughout the year in Dorrigo.

The progress of infection with Theilerial genotypes in the 30 introductions were similar to earlier reports in Japan (Ota et al., 2009) and NSW (Eamens et al., 2013a; Jenkins et al. 2015). In the latter study, 10 cows introduced in to an endemic Theilerial region were found sequentially infected with *T.orientalis* ikeda and chitose and latterly and to a lesser extent, with *T.orientalis* buffeli over 2.5 months. One cow developed anaemia, but effects on productivity were not examined. However, in the current study, 3 animals died within the larger bovine cohort (1% mortality) in the first 2 months after arrival, but the parasitosis with reduced PCV and ADLG incurred > 20Kg productivity loss per animal over the first 3 months at Dorrigo (Fig. 4). In New Zealand, Lawrence et al., (2019b) also reported that the weight gains of calves infested with *T.orientalis* ikeda were significantly reduced by 0.13Kg/day over an 8–10 week period, amounting to around 10Kg weight loss.

After this “first wave “of parasitism, the parasitosis subsided into the carrier state after 3 months and remained relatively stable for the following 3 months to August. Over the same 3-month period, the PCV and ADLG recovered to pre-infection levels of around 32 and 0.8 kg/day, respectively, but the initial weight loss caused by Theilerial infestation had not been recovered by the end of winter, some 6 months after

arrival. These observations from 3–6 months (after recovery from the initial wave of Theilerial parasitosis) were consistent with the “normal” performance recorded for Theilerial carriers (Perera et al., 2014; Lawrence et al., 2019a). In this “carrier” state, the concentrations of DNA from *T.orientalis* genotypes ikeda and chitose were reduced to similar levels exemplified by the buffeli genotype throughout the monitoring period. This result tantalisingly suggests some regulatory mechanism of this particular level of parasitosis and possibly explains why *T.orientalis* buffeli is considered “benign”. A carrier state with minimal effect on productivity would certainly be advantageous to the parasite, allowing uptake by 3 host-ticks which have to renew infestation in each generation before sexual reproduction and transmission of Theileria can recur.

The reasons for changes in the population dynamics of Theilerial genotypes are speculative as the host immune response to *T. orientalis* infection and the genotypic interactions within the Theilerial merozoite populations in cattle are not completely understood. However, the development of carrier states with low levels of genotypic GC/ul are attained whether host cattle are infested with single ikeda (Hammer et al., 2016) or multiple Theilerial genotypes (Jenkins et al., 2015; this study). Population changes during co-infection with multiple genotypes could mimic the strain-specific cell-mediated responses seen with *T.annulata* and *T.parva* (Nene & Morrison, 2016) or less likely, genetic recombination within the tick vector as seen in another apicomplexan, *Plasmodium falciparum*, within mosquitos (Matthews et al., 2018). In the case of malaria, genotypic changes establish a chronic parasitosis that impairs the establishment of subsequent infections which are arrested in the liver (Portugal et al., 2016). Thus older human carriers have a reduced incidence of clinical infection, often harbouring subclinical infestations with multiple parasite genotypes (Portugal et al., 2016). This scenario is analogous to the lack of clinical disease in Theilerial carriers and does accord with the findings of Jenkins et al.(2015). Here, in 10 introduced cattle, a phylogenetic cluster of the *T.orientalis* chitose A genotype and the ikeda genotype were associated with clinical disease, with chitose the major genotype following the first wave of parasitosis (days 50–75; Jenkins et al., 2015). The genotypic parasitoses in the Dorrigo cattle did not conform to the same pattern, but the 3 Theilerial genotypes (ikeda, chitose and buffeli) were each present at similar levels of GC/ul through the carrier state which established from 3–6 months after arrival. Whether this outcome resulted from a maturing host response or an inherent property of *T.orientalis* akin to helminth hypobiosis is not known. As Theileriosis can recrudesce in carrier cattle under stresses such as long distance transport, especially in advanced pregnancy (Eamens et al., 2013b), this effect strongly suggests an active component of host regulation on parasite populations, analogous to the “peri-parturient relaxation of immunity” for nematode parasites.

Overall, the results also suggest that if the impact of this initial parasitosis with the virulent genotypes of *T.orientalis* can be more effectively managed or reduced, productivity losses may be mitigated to some extent. Because of the lack of clinical Theileriosis in Queensland, Australia, which has endemic *T.orientalis* buffeli, (Stewart et al., 1992) together with the lack of recrudescence in the vast majority of Theilerial carriers in endemic zones, prior immunisation with *T.orientalis* buffeli has been suggested as a “vaccine” inducing “premunity”(Neal et al., 1969) against the virulent genotypes (de Vos, 2011). This approach was shown to be effective in controlling the initial wave of parasitosis after tick challenge with

T.orientalis ikeda (Emery et al., submitted), raising the possibility of a preventive measure for agistment of cattle.

5. Conclusions

In endemic region of *T.orientalis*, calves and introduced cattle are most at risk of developing clinical signs with death and production losses. At Dorrigo, NSW, this study confirmed and extended previous reports, finding that all naïve calves and introduced weaners became PCR-positive for *T.orientalis* genotypes ikeda and chitose with 3 weeks. Maximal parasitoses were attained around 5-8 weeks after birth or introduction, before declining as animals recovered and enter the carrier state. In the introduced cohort, the first wave of parasitosis caused around 20Kg weight loss. In contrast, parasitosis with *T.orientalis* buffeli occurred slowly in an increasing proportion of the test groups over time, with gene copies of the parasite only attaining the “resting levels” of parasitism displayed by the virulent genotypes after the first wave of parasitosis. The results imply that controlling the first wave of ikeda and chitose parasitism is key to reducing deaths and production losses.

Declarations

Competing interests. The authors declare that they have no competing interests

Acknowledgements.

This project was funded by Meat & Livestock Australia (MLA), Project PPSH.0832. The expert assistance of Dr Evelyn Hall (statistics), Ms Maira Meggiolaro (PCR) and University Staff at Pye Farm are all much appreciated.

References

1. Bogema DR, Deutscher AT, Fell S, Collins D, Eamens GJ, Jenkins C. Development and Validation of a Quantitative PCR Assay Using Multiplexed Hydrolysis Probes for Detection and Quantification of *Theileria orientalis* Isolates and Differentiation of Clinically Relevant Subtypes. J. Clin. Microbiol. 2015; 53: 941-950.
2. De Vos AJ. Theileria: Assess potential to develop a vaccine for *Theileria orientalis* infection. 2011. MLA report B.AHE.0076.
3. Eamens GJ, Bailey G, Gonsalves JR, Jenkins C. Distribution and temporal prevalence of *Theileria orientalis* major piroplasm surface protein types in eastern Australian cattle herds. Aust. Vet. J. 2013a; 8: 332-340.
4. Eamens GJ, Gonsalves JR, Jenkins C, Collins D, Bailey G. *Theileria orientalis* MPSP types in Australian cattle herds associated with outbreaks of clinical disease and their association with clinical pathology findings. Vet. Parasitol. 2013b;191: 209-17.

5. Emery DL, de Burgh S, Dinh THHH, Rolls P, Carter P. Merozoites of *Theileria orientalis* buffeli reduce parasitosis following challenge by ticks infested with *T.orientalis* ikeda. (2020; submitted)
6. Hammer JF, Emery D, Bogema D, Jenkins C. Detection of *Theileria orientalis* genotypes in *Haemaphysalis longicornis* ticks from southern Australia. *Parasit. Vectors* 2015; 8, 229. <https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-015-0839-9>.
7. Hammer JF, Bogema D, Jenkins C, Emery D. Mechanical transfer of *Theileria orientalis*: possible roles of biting arthropods, colostrum and husbandry practices in disease transmission. *Parasit. Vectors*. 2016; 9: 34-39.
8. Izzo MM, Poe I, Horadagoda N, De Vos AJ, House J K. Haemolytic anaemia in cattle in NSW associated with *Theileria* infection. *Aust. Vet. J.* 2010; 88: 45–51.
9. Jenkins C, Micallef M, Alex S, Collins , Djordevic, SP, Bogema DR. Temporal dynamics and subpopulation analysis of *Theileria orientalis* genotypes in cattle. *Infection, Genet. Evol.* 2015; 32: 199-207.
10. Kamau J, de Vos AJ, Playford M, Salim B, Kinyanjui P, Sugimoto C. Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks. *Parasit. Vectors* 2011; 4: 22-31.
11. Kawazu S, Sato , Kamio, T, Yokomizo Y, Fujisaki K, Honma S, Minami T. Immunohistological staining for detection of *Theileria sergenti* schizonts. *J. Vet. Med. Sci.* 1991; 53: 743-745.
12. Lawrence KE, Gedye K, Pomro W.E. A longitudinal study of the effect of *Theileria orientalis* Ikeda type infection on three New Zealand dairy farms naturally infected at pasture. *Vet. Parasitol.* 2019a; 276, 108977. DOI: 10.1016/j.vetpar.2019.108977.
13. Lawrence KE, Lawrence BL, Hickson RE, Hewitt CA, Gedy K, Fermin LM, Pomro W.E. Associations between *Theileria orientalis* Ikeda type infection and the growth rates and haematocrit of suckled beef calves in the North Island of New Zealand. *N.Z. Vet. J.* 2019b; 67: 66- 73.
14. Marendy D, Baker K, Emery D, Rolls P, Sutchbury R. *Haemaphysalis longicornis*: the life-cycle on dogs and cattle, with confirmation of its vector status for *Theileria orientalis* in Australia. *Parasit. Vectors*. 2019; <https://doi.org/10.1016/j.vpoa.2019.100022>
15. McFadden AMJ, Rawdon TG, Mayer J, Makin, , Morley, M, Clough R, Tham, K, Mullner P, Geysen D. An outbreak of haemolytic anaemia associated with infection of *Theileria orientalis* in naïve cattle. *N.Z. Vet. J.* 2011; 59:79–85.
16. Matthews H, Duffy CW, Merrick CJ. Checks and balances? DNA replication and the cell cycle in *Plasmodium*. *Parasit. Vectors* 2018;11: 216- 222. <https://doi.org/10.1186/s13071-018-2800-1>
17. Neal RA, Garnham, PCC, Cohen, S. Immunisation against protozoal parasites. *Brit. Med. Bull.* 1969; 25: 194-210.
18. Nene V, Morrison WI. Approaches to vaccination against *Theileria parva* and *Theileria annulata*. *Parasite Immunol.* 2016; 38: 724-734. doi:10.1111/pim.12388
19. Ota N, Mizuno D, Kuboki N, Igarashi I, Nakamura Y, Yamashin H, Hanzaike, T, Fujii K, Onoe S, Hata H, Kondo S, Matsui S, Koga M, Matsumoto K, Inokuma H, Yokoyama N. Epidemiological survey of

- Theileria orientalis* infection in grazing cattle in the eastern part of Hokkaido, Japan. Jap.J. Vet. Med. Sci. 2009; 71: 937–944.
20. Park J, Han Y, Han, D. *et al.* Genetic characterization of *Theileria orientalis* from cattle in the Republic of Korea. Parasitol. Res. 2017; 116:449–454. <https://doi.org/10.1007/s00436-016-5316-7>.
 21. Perera PK, Gasser R, Firestone, SM, Anderson GA, Malmo J, Davis G, Beggs .S. Jabbar A. Oriental theileriosis in dairy cows causes a significant milk production loss. Parasit Vectors. 2014; 7: 73-81.
 22. Portugal S, Carret C, Recker M, Armitage AE, Gonçalves LA, Ephiphany S, Sullivan , Roy C, Newbold C, Drakesmith C, Mota MM. Host mediated regulation of superinfection in malaria. *Nature Med.* 2011; 17: 732-737.
 23. Reik RF. Epidemiology and transmission of *Theileria* sp of cattle in Australia. Aust. Vet. J. 1982; 59: 89-92.
 24. Sivakumar T, Hayashida K, Sugimoto C, Yokoyama N. Evolution and genetic diversity of *Theileria*. Inf. Genet. Evol.2014; 27: 250–263.
 25. Skilton RA, Bishop RP, Katende JM, Mwaura S, Morzaria SP. The persistence of *Theileria parva* infection in cattle immunized using two stocks which differ in their ability to induce a carrier state: analysis using a novel blood spot PCR assay. Parasitology. 2002; 124: 265–276.
 26. Stewart NP, Standfast N.F, Baldock FC, Reid DJ, DeVos AJ. The distribution and prevalence of *Theileria buffeli* in cattle in Queensland. Aust. Vet. J. 1992; 69: 59-61.
 27. Stewart N, de Vos AJ, McGregor W, Shiels I. The experimental transmission of *Theileria buffeli* of cattle in Australia by *Haemaphysalis humerosa*. Aust. Vet .J. 1987a; 64: 81-3.
 28. Stewart NP, de Vos AJ, McGregor W, Shiels, I. *Haemaphysalis humerosa*, not *H. longicornis*, is the likely vector of *Theileria buffeli* in Australia. Aust. Vet. J. 1987b; 64: 280-282. doi:10.1111/j.1751-0813.1987.tb15960.x
 29. Swilks E, Fell SS, Hammer JF, Sales, N, Krebs L, Jenkins, C. Transplacental transmission of *Theileria orientalis* occurs at a low rate in field affected cattle: infection in utero does not appear to be a major cause of abortion. Parasite Vectors. 2017;10: 227-236.

Figures

Figure 1

Effect of age on parasitoses with *T.orientalis* genotypes (chitose, ikeda, buffeli) for 111 calves born at Dorrigo in Spring 2017 and 2019. The letters indicate the significance of the age on the genotypes (colour coded), Significant age-related differences ($p < 0.05$) are indicated with different superscripts for genotypes chitose (a-c); ikeda (d-f) and buffeli (g-i).

Figure 2

Parasitosis of *Theileria* genotypes ikeda (black), chitose (red) and buffeli (blue) expressed as gene copies per ul blood (GC/ul) in 90 calves born at Dorrigo in Spring 2017 (left) and 60 calves born in Spring 2019 (right). Comparisons include: (A), Spring 2017, ikeda and chitose (top left); (B), Spring 2019, ikeda and chitose (top right); (C), Spring 2017, buffeli (bottom left); (D), Spring 2019, buffeli (bottom right). Lines of best fit are displayed into each section.

Figure 3

Kinetics of parasitosis by *Theileria* genotypes *ikeda* (solid line), *buffeli* (short dashes) and *chitose* (long dashes), expressed as mean GC/uL blood in the first 6 months after 30 cattle were moved to Dorrigo in Feb 2020. The haematocrit (PCV; dotted lines) is also displayed.

Figure 4

Progressive actual (short dashed line) and expected weight gains (long dashed line) of weaner cattle after introduction to Dorrigo in February 2020. The average daily liveweight gain (ADLG; ; solid line) is also displayed.