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BRIEF REPORT

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Theileria orientalis Ikeda infection detected in red deer but not dogs or horses in New Zealand

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ABSTRACT

Aims: To determine whether evidence for infection with *Theileria orientalis* (Ikeda) could be identified in samples of commercial red deer *(Cervus elaphus)*, horses, and working farm dogs in New Zealand.

Methods: Blood samples were collected during October and November 2019 from a convenience sample of red deer (n = 57) at slaughter. Equine blood samples (n = 50) were convenience-sampled from those submitted to a veterinary pathology laboratory for routine testing in January 2020. Blood samples, collected for a previous study from a convenience sample of Huntaway dogs (n = 115) from rural regions throughout the North and South Islands of New Zealand between August 2018 and December 2020, were also tested. DNA was extracted and quantitative PCR was used to detect the *T. orientalis* Ikeda major piroplasm surface protein (MPSP) gene. A standard curve of five serial 10-fold dilutions of a plasmid carrying a fragment of the *T. orientalis* MPSP gene was used to quantify the number of *T. orientalis* organisms in the samples. MPSP amplicons obtained by end-point PCR on positive samples were isolated and subjected to DNA sequencing. The resulting sequences were compared to previously published *T. orientalis* sequences.

Results: There were 6/57 (10%) samples positive for *T. orientalis* Ikeda from the deer and no samples positive for *T. orientalis* Ikeda from the working dogs or horses. The mean infection intensity for the six PCR-positive deer was 5.1 (min 2.2, max 12.4) *T. orientalis* Ikeda organisms/µL.

Conclusions and clinical relevance: Red deer can potentially sustain low infection intensities of *T. orientalis* Ikeda and could act as reservoirs of infected ticks. Further studies are needed to determine whether naïve ticks feeding on infected red deer can themselves become infected.

Abbreviations: Cq: Quantification cycle; LOQ: Limits of quantification; MPSP: Major piroplasm surface protein; qPCR: Quantitative polymerase chain reaction

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Introduction

In August 2012, an outbreak of bovine theileriosis, associated with the apicomplexan blood parasite *Theileria orientalis* Ikeda, was first diagnosed in New Zealand (Pulford *et al*. 2016a). Since then, the disease has spread quickly, becoming endemic throughout much of the North Island and parts of the upper South Island, matching the distribution of the New Zealand cattle tick, *Haemaphysalis longicornis*, which is the sole vector for the parasite (Heath 2016). *Theileria orientalis* Ikeda itself belongs to a branch of the *Theileria* genus known as the non-transforming *Theileria* (Watts *et al*. 2016). Other members of this non-transforming group include *T. orientalis* Chitose and *T. orientalis* Buffeli, and although the non-transforming *Theileria* are generally considered benign, causing fewer symptoms in infected cattle than the transforming *Theileria*, the Ikeda type has been more often associated with severe outbreaks of bovine anaemia, jaundice, loss of production, abortion and death (Lawrence *et al*. 2016a; Oakes *et al*. 2019).

Although cattle are the preferred host, Lawrence *et al*. (2021) found that sheep could potentially maintain infections with low numbers of *T. orientalis* Ikeda and that naïve *H. longicornis* larvae became infected after feeding on infected sheep. Similarly, there is some evidence from overseas that dogs could potentially act as a reservoir for *T. orientalis* infection (Bawm *et al*. 2021). The only studies that have been conducted in deer are in white tailed deer (*Odocoileus virginianus*) and sika deer (*Cervus nippon yesoensis*) and neither of these found evidence of infection (Shibata *et al*. 2018; Thompson *et al*. 2022). No research investigating whether horses can

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be infected with *T. orientalis* could be identified by the authors.

The aim of this study was to determine whether evidence for infection with *Theileria orientalis* Ikeda could be identified in samples from commercial red deer (*Cervus elaphus*), horses and working farm dogs in New Zealand.

Materials and methods

Deer samples

A total of 57 blood samples were collected as a convenience sample from two lines of deer slaughtered at Duncan New Zealand Ltd. (Rotorua, NZ). The first samples were from 47 deer from a single deer farm in Reporoa, slaughtered on 24 October 2019, and the second samples were from 10 deer from a single deer farm in Pukekohe, slaughtered on 21 November 2019. The second group of deer were observed to have heavy infestations of ticks when processed at the abattoir. No details of sex or age were recorded at sampling apart from the observation that the deer were well grown. There was no available information on the tick density on the farms from which the deer originated, or whether the sampled deer had previously been treated with a tickicide. Blood was collected into EDTA tubes as a free-flowing catch when the dead deer were hoisted and bled. Samples were transported to Massey University (Palmerston North, NZ) chilled and were stored at −20°C prior to DNA extraction. Deer were the only animals slaughtered at this abattoir so there was no opportunity for cross-contamination from cattle or sheep.

Equine samples

A convenience sample of 50 anonymised blood samples from horses in the upper North Island was supplied by SVS Laboratories Ltd. (Hamilton, NZ, as per companies terms of trade). These blood samples, which were mostly from racehorses and some foals, were all the equine samples of whole blood collected into EDTA tubes that were submitted to SVS Laboratories for routine complete blood count analysis between 10 and 17 January 2020. After analysis at SVS, the blood samples were refrigerated before being sent to Massey University in chilled containers on 17 January 2020.

Canine samples

There were 115 canine blood samples, which were originally opportunistically collected as part of a different study into the prevalence of the ABCB1-1Δ gene mutation in Huntaway dogs between January 2019 and April 2021 (Gedye *et al*. 2023). The Huntaway is a recognised working breed on New Zealand sheep and beef farms, and the sampled dogs were from rural properties in the Gisborne, Waikato, Manawatū/Whanganui, Hawke's Bay, Canterbury, and Otago regions. Blood was collected into vacutainers containing EDTA and stored at −20°C prior to DNA extraction. A few of the dogs sampled were from Ranfurly and Christchurch (in the South Island, but outside of the accepted tick areas of New Zealand) but were left in the study as negative controls. The blood collections were approved by the Massey University Animal Ethics Committee (approval numbers 18/27 and amendment 18/211) and owners consented to samples being stored and used for future research. The enrolled dogs were mostly between 2 and 10 years of age with a few younger.

Laboratory methods

DNA extraction

DNA was extracted from 50 µL of the collected EDTA blood using the NucleoMag Vet kit (Machery-Nagel, Düren, Germany) processed on a KingFisher Flex System (Thermo Fisher, Waltham, MA, USA). Extracted DNA was quantified using the Qubit dsDNA HS Assay Kit and Qubit 2.0 Fluorometer (Thermo Fisher). Extracted DNA was stored at −80°C until analysis. The DNA extracted from 50 µL of whole blood was eluted in 100 µL PCR-grade water and then 5 µL was pipetted for the PCR analysis.

Quantitative PCR

Quantitative PCR (qPCR) was performed on the extracted DNA using the primers and probe previously described for *T. orientalis* Ikeda by Pulford *et al*. (2016b). All primers and probes were sourced from Integrated DNA Technologies (Coralville, IA, USA). qPCR was performed with the following conditions: 1 x PerfeCTa qPCR ToughMix (Quantabio, Beverly, MA, 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 Mic qPCR thermal cycler (BioMolecular Systems, Upper Coomera, QLD, Australia). Thermal cycling conditions were as follows: 95°C for 2 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds, with fluorescence capture on the 60°C step. Standard curves (100 pg/µL to 10 fg/µL) were produced using a serial dilution of a plasmid from a previously produced clone of a section of the *T. orientalis* Ikeda major piroplasm surface protein (MPSP) gene (provided by author KG). Runs were analysed using the thermocycler software and exported for further statistical analysis. The standards were also the positive controls, with negative controls being PCR-grade water. All PCR testing was carried out in triplicate. For the first screening rounds, no

Ikeda plasmid DNA standards were included, to prevent cross contamination. In the second round, any PCR-positive samples were re-analysed with Ikeda plasmid DNA standards included.

Gene copies and validation of assay performance

The number of copies of the Ikeda MPSP gene in a blood sample was quantified by multiplying the molarity of the *Theileria* DNA in the blood sample, as calculated from the standard curve using the quantification cycle (Cq) value for the PCR amplification, by Avogadro's number (6.022×10^{23}). A previous unrelated study had estimated the limits of quantification (LOQ) for this molecular assay as 1 fg/µL (219 organisms/µL) (Lawrence *et al*. 2018). While it is still possible to estimate a value below the LOQ, this is given with much less confidence than when above the LOQ.

Gene sequencing of T. orientalis *types*

Positive qPCR samples were analysed for the genotype of *T. orientalis* via an end-point PCR using previously described primers (Pulford *et al*. 2016b), which also amplify the MPSP gene. The PCR reaction was performed as follows: 1 x KAPA HiFi Buffer (KAPA Biosystems, Wilmington, MA, USA), 0.3 mM dNTP, 300 nM of each primer (Integrated DNA Technologies), 0.5 U KAPA HiFi DNA polymerase (KAPA Biosystems) and 2 µL template, in a final reaction volume of 25 µL. Thermal cycling conditions were as follows: 5 minutes at 95°C, 40 cycles of 98°C for 20 seconds, 60°C for 15 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. All thermal cycling was performed in a SensoQuest Labcycler (SensoQuest, Göttingen, Germany). Negative (water) and positive (clone of the Ikeda genotype) controls were used for all PCR. PCR amplicons were separated in a 1% w/v agarose (Bioline, London, UK) gel with 0.5 x Tris/borate/EDTA buffer and visualised under ultraviolet light. Amplicons were compared for size to a 100 bp DNA ladder (Hyperladder; Bioline), bands of the appropriate size were cut from the gel, eluted overnight in elution buffer (10 mM Tris-HCl, pH 8) and sent for bi-directional Sanger sequencing to Massey Genome Services (Massey University). Sequences were compared to previously published *T. orientalis* sequences from the National Centre for Biotechnology Information[1] specifically Ikeda (AB469170), Chitose (AB469169) and Buffeli (AB520945), using Geneious v10.1.3 (GraphPad Software, Boston, MA, USA; Kearse *et al*. 2012).

Results

Of the 57 blood samples from red deer, 6/57 (10%) were weakly positive (Cq > 30) for *T. orientalis* Ikeda type; however, none had an infection intensity > LOQ. Five of these red deer were from the Reporoa deer farm (5/47, 10%) and one was from the Pukekohe deer farm (1/10; 10%). The mean infection intensity for the six PCR-positive red deer was 5.1 (min 2.2, max 12.4) Ikeda organisms/µL. Three deer samples were selected for end-point PCR but only one amplified an appropriately sized amplicon. Comparison to previously published *T. orientalis* sequences (Pulford *et al*. 2016b) confirmed that this deer was infected with *T. orientalis* Ikeda. The deer was from the Reporoa deer farm, and the infection intensity was 12.4 Ikeda organisms/µL.

No blood samples from dogs or horses were positive for *T. orientalis* Ikeda DNA.

Discussion

This study shows that it is possible for a proportion of farmed red deer to carry very low infections of *T. orientalis* Ikeda type, and although the infection intensities measured were well below the LOQ for this assay, they were like those previously found for naturally infected ewes (mean 2.7 (min 0.01, max 18.9) Ikeda organisms/µL), and ewe-lambs (mean 8.1 (min 2.8, max 25.5) Ikeda organisms/µL) (Lawrence *et al*. 2021). Furthermore, the infection intensities found in sheep were sufficient to infect naïve *H. longicornis* larvae that fed on them (Lawrence *et al*. 2021). There was no evidence, however, that horses or working farm dogs can become infected with *T. orientalis* Ikeda.

The best time to sample cattle for evidence of *T. orientalis* infection is about 4 weeks after the peak in nymph or adult *H. longicornis* feeding activity, i.e. late spring or early summer, respectively. This should result in the highest infection intensities for naïve cattle, > 300,000 Ikeda organisms/µL, if the feeding ticks are themselves infected. Once past this acute phase, the infection intensity of cattle will drop to below 10,000 Ikeda organisms/µL, even if these same cattle continue to have infected ticks feed on them (Lawrence *et al*. 2019). This likely means that for most of the research subjects, sampling was not at the optimum time to identify acute infection intensities as the deer may have been infected as fawns the previous year. However, it was our belief that animals that had previously been infected with *T. orientalis* Ikeda would carry a chronic, low-intensity infection for an extended period, which this study could have found. As this was only a pilot study, the finding of only one or two infected horses or dogs would have been significant. To determine with certainty whether dogs or horses can be infected with *T. orientalis* Ikeda would require repeatedly sampling young horses or young dogs

[1] www.ncbi.nlm.nih.gov

from areas with endemic theileriosis, over the peak tick feeding periods. Furthermore, to establish whether deer can transmit infection to naïve ticks would require that feeding experiments be conducted similar to those completed in sheep by Lawrence *et al*. (2021).

Clinical signs have been reported in 95% of cattle with an infection intensity > 300,000 Ikeda organisms/μL (Bogema *et al*. 2015). The infection intensities recorded in the sampled deer in this study were much lower than those seen in diseased cattle and thus are likely to be asymptomatic. However, the finding that deer may be able to maintain an active *T. orientalis* Ikeda infection, meaning that naïve ticks that feed on deer can potentially become infected, may explain why the density of deer farms was an important covariant in a maximum entropy model developed to predict the relative environmental suitability for *T. orientalis* transmission across New Zealand (Lawrence *et al*. 2016b). In Japan, deer have long been considered an amplifier species for ticks (Tsukada *et al*. 2014), and in New Zealand, red deer have been identified as a key sentinel species for tick surveillance (McFadden *et al*. 2016), which was the reason for including deer farm density in the maximum entropy model.

As sheep in New Zealand have also been shown to maintain active infection, the impact of infected deer on the epidemiology of oriental theileriosis may not be so great here. However, in countries that have few sheep but large populations of feral red deer, and where cattle farms frequently border habitats of feral red deer, the impact may be much greater.

The prevalence of *T. orientalis* Ikeda infection in the sampled deer in this study was 10%, which was much lower than the prevalence of infection found in naturally infected sheep of 50–78% (Lawrence *et al*. 2021). However, many of the sheep from that study were sampled on a farm with a very high density of ticks, whereas there was no available information on the tick density on the farms from which the deer in this study originated or whether the sampled deer themselves had previously been treated with a tickicide. The use of tickicides could potentially have adversely affected the results of this study, although the second line of deer were observed to be heavily infested with ticks at slaughter. The finding that both lines of deer had the same proportion of infected deer (~ 10%) is difficult to explain and will need further study to develop a plausible explanation.

The finding that no horses or dogs were infected was reassuring and probably not surprising given that there have been no reports of the disease in these species in the 12 years since *T. orientalis* Ikeda infection first arrived in New Zealand. There have been no reports of the disease in sheep or deer either, but as dogs and horses are companion animals and subject to a far greater level of individual handling, care, and scrutiny, then it seems likely that even minor symptoms in these species would have been observed by now.

Conclusion

Commercial red deer can potentially sustain low infection intensities of *T. orientalis* Ikeda infection, which could be important in the epidemiology of evolving epidemics in countries with few sheep, like the current outbreak in the USA.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

Bawm S, Myaing TT, Thu MJ, Akter S, Htun LL, Win MM, Nonaka N, Nakao R, Katakura K. PCR detection and genetic characterization of piroplasms from dogs in Myanmar, and a possible role of dogs as reservoirs for *Theileria* parasites infecting cattle, water buffaloes, and goats. *Ticks and Tick-borne Diseases* 12, 101729, 2021. https://doi.org/10.1016/j.ttbdis.2021.101729

Bogema R, Deutscher A, Fell S, Collins D, Eamens G, Jenkins C. Development and validation of a multiplexed hydrolysis probe qPCR assay for the detection and quantification of *Theileria orientalis* and differentiation of clinically-relevant subtypes. *Journal of Clinical Microbiology* 53, 2015. https://doi.org/10.1128/JCM.03387-14

Gedye K, Poole-Crowe E, Shepherd M, Wilding A, Parton K, Lopez-Villalobos N, Cave N. Prevalence of the ABCB1-1Δ gene mutation in a sample of New Zealand Huntaway dogs. *New Zealand Veterinary Journal* 71, 133–6, 2023. https://doi.org/10.1080/00480169.2023.2181238

Heath A. Biology, ecology and distribution of the tick, *Haemaphysalis longicornis* Neumann (Acari: Ixodidae) in New Zealand. *New Zealand Veterinary Journal* 64, 10–20, 2016. https://doi.org/10.1080/00480169.2015.1035769

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C. Geneious Basic: an integrated and extendable desktop software platform for the organization and

analysis of sequence data. *Bioinformatics* 28, 1647–9, 2012. https://doi.org/10.1093/bioinformatics/bts199

Lawrence K, McFadden A, Gias E, Pulford D, Pomroy W. Epidemiology of the epidemic of bovine anaemia associated with *Theileria orientalis* (Ikeda) between August 2012 and March 2014. *New Zealand Veterinary Journal* 64, 38–47, 2016a. https://doi.org/10.1080/00480169.2015.1090894

Lawrence K, Summers S, Heath A, McFadden A, Pulford D, Pomroy W. Predicting the potential environmental suitability for *Theileria orientalis* transmission in New Zealand cattle using maximum entropy niche modelling. *Veterinary Parasitology* 224, 82–91, 2016b. https://doi.org/10.1016/j.vetpar.2016.05.018

Lawrence K, Gibson M, Hickson R, Gedye K, Hoogenboom A, Fermin L, Draganova I, Pomroy W. Experimental infection of Friesian bulls with *Theileria orientalis* (Ikeda) and effects on the haematocrit, live weight, rectal temperature and activity. *Veterinary Parasitology: Regional Studies and Reports* 14, 85–93, 2018. https://doi.org/10.1016/j.vprsr.2018.09.004

Lawrence K, Gedye K, Pomroy W. A longitudinal study of the effect of *Theileria orientalis* Ikeda type infection on three New Zealand dairy farms naturally infected at pasture. *Veterinary Parasitology* 276, 108977, 2019. https://doi.org/10.1016/j.vetpar.2019.108977

Lawrence K, Gedye K, Hickson R, Wang B, Carvalho L, Zhao Y, Pomroy W. The role of sheep (*Ovis aries*) in maintaining *Theileria orientalis* Ikeda type infection. *Veterinary Parasitology* 291, 109391, 2021. https://doi.org/10.1016/j.vetpar.2021.109391

McFadden A, Vink D, Pulford D, Lawrence K, Gias E, Heath A, McFadden C, Bingham P. Monitoring an epidemic of *Theileria*-associated bovine anaemia (Ikeda) in cattle herds in New Zealand. *Preventive Veterinary Medicine* 125, 31–7, 2016. https://doi.org/10.1016/j.prevetmed.2015.11.005

Oakes VJ, Yabsley MJ, Schwartz D, LeRoith T, Bissett C, Broaddus C, Schlater JL, Todd SM, Boes KM, Brookhart M. *Theileria orientalis* Ikeda genotype in cattle, Virginia, USA. *Emerging Infectious Diseases* 25, 1653–9, 2019. https://doi.org/10.3201/eid2509.190088

Pulford D, McFadden A, Hamilton J, Donald J. Investigation of the index case herd and identification of the genotypes of *Theileria orientalis* associated with outbreaks of bovine anaemia in New Zealand in 2012. *New Zealand Veterinary Journal* 64, 21–8, 2016a. https://doi.org/10.1080/00480169.2015.1090355

Pulford D, Gias E, Bueno I, McFadden A. Developing high throughput quantitative PCR assays for diagnosing Ikeda and other *Theileria orientalis* types common to New Zealand in bovine blood samples. *New Zealand Veterinary Journal* 64, 29–37, 2016b. https://doi.org/10.1080/00480169.2015.1089798

Shibata S, Sivakumar T, Igarashi I, Umemiya-Shirafuji R, Inokuma H, Fukumoto S, Yokoyama N. Epidemiological survey of a cervine *Theileria* in wild deer, questing ticks, and cattle in Hokkaido, Japan. *Ticks and Tick-borne Diseases* 9, 1235–40, 2018. https://doi.org/10.1016/j.ttbdis.2018.05.006

Thompson AT, Garrett KB, Kirchgessner M, Ruder MG, Yabsley MJ. A survey of piroplasms in white-tailed deer (*Odocoileus virginianus*) in the southeastern United States to determine their possible role as *Theileria orientalis* hosts. *International Journal for Parasitology: Parasites and Wildlife* 18, 180–3, 2022. https://doi.org/10.1016/j.ijppaw.2022.05.005

Tsukada H, Nakamura Y, Kamio T, Inokuma H, Hanafusa Y, Matsuda N, Maruyama T, Ohba T, Nagata K. Higher sika deer density is associated with higher local abundance of *Haemaphysalis longicornis* nymphs and adults but not larvae in central Japan. *Bulletin of Entomological Research* 104, 19–28, 2014. https://doi.org/10.1017/S0007485313000308

Watts J, Playford M, Hickey K. *Theileria orientalis*: a review. *New Zealand Veterinary Journal* 64, 3–9, 2016. https://doi.org/10.1080/00480169.2015.1064792