Developing high throughput quantitative PCR assays for diagnosing Ikeda and other *Theileria orientalis* types common to New Zealand in bovine blood samples

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Supplementary Figure 1. Mapping PCR oligonucleotide primers and probes designed for quantitative PCR to a CLUSTALW multiple alignment of major piroplasm surface protein nucleotide sequences from GenBank or collected from New Zealand samples for *Theileria orientalis* Ikeda, Chitose and Buffeli types.

Genotype consensus sequences for New Zealand field samples were generated from raw MPSP (Ota *et al.* 2011) amplicon nucleotide sequences if they had no ambiguous base calls in the raw data, and they were positive by conventional PCR to only one *T. orientalis* type. Consensus sequences for Ikeda (NZ Ikeda Con) were generated from KP795979, KP795981, KP795982, for Buffeli (NZ Buffeli Con) from KP795978 and KP795983 using CLUSTALW, and for Chitose (NZ Chitose Con) KP795977 (Pulford *et al.* 2015). A multiple-alignment of New Zealand type consensus sequences with reference sequences for type 1 Chitose (THETS32KC), type 2 Ikeda (THEPMIPI) and type 3 Buffeli (THEPMIPW) was generated with CLUSTALW. Nucleotide positions shared with the consensus sequence are shown as dots, otherwise variations from the consensus are shown with a single letter code polymorphism. Primer and probe positions are shown as arrows, Th.ori primers are shown in green and grey, type specific qPCR primers are shown in shades of green, and probes in red.

Ota N, Mizuno D, Kuboki N, Igarashi I, Nakamura Y, Yamashina H, Hanzaike T, Fujii K, Onoe S, Hata H *et al.* Epidemiological survey of *Theileria orientalis* infection in grazing cattle in the eastern part of Hokkaido, Japan. *Journal of Veterinary Medical Science* 71, 937–44, 2009

Pulford DJ, McFadden, AMJ, Hamilton, JS, Donald J. Investigation of the index case herd and identification of the strain types of *Theileria orientalis* associated with outbreaks of bovine anaemia in New Zealand in 2012. *New Zealand Veterinary Journal* doi: 10.1080/00480169.2015.1090355, 2015

Supplementary Information

S2

[illegible]

Supplementary Figure 2. The copy number of *Theileria orientalis* Ikeda genomes in blood measured using an Ikeda-specific quantitative PCR. Samples shown were obtained from a dairy herd in Ruawai, Northland from cattle with clinical signs of infection with *T. orientalis*.