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Original article

Experimental infection of Friesian bulls with *Theileria orientalis* (Ikeda) and effects on the haematocrit, live weight, rectal temperature and activity

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ABSTRACT

Since 2012, New Zealand has suffered from an epidemic of infectious bovine anaemia associated with *T. orientalis* (Ikeda), an obligate intracellular protozoan parasite of cattle. Despite widespread agreement that *T. orientalis* (Ikeda) infection has impacted beef and dairy farming in New Zealand there is very little quantitative data to support this conclusion. A randomised controlled experimental study of the effect of *T. orientalis* (Ikeda) infection on the live weight, haematocrit (HCT), temperature and activity of 2-year-old Friesian bulls was conducted at a Massey University Research farm, Palmerston North. Ten out of seventeen 2-year-old Friesian bulls were injected intravenously with 30 mL whole blood from 2 clinical cases of *Theileria*-associated bovine anaemia and then followed over a period of 20 weeks. The bulls were blood sampled, had rectal temperature recorded and weighed 3 times weekly for 13 weeks and then once weekly thereafter until the end of the trial. Infection intensity was monitored using qPCR.

All 10 inoculated bulls were successfully infected with *T. orientalis* (Ikeda). The results showed that the live weight response to infection was varied and the bulls could be divided into two groups based on this response. Four infected bulls showed a significant weight reduction of 41.5 kg ($p < 0.0001$), a financial loss of around NZ $112 per bull, compared with the other 6 bulls in the infected group, which were not different to the 7 uninfected controls. The live weight of the 4 poor growing bulls was significantly lower than the other 6 infected bulls from Day 71 post infection ($p < 0.05$).

All ten infected bulls showed a similar decrease in HCT, with the lowest HCT reached around Day 60 to 80 post-infection, however the four infected bulls that grew poorly did have a significantly elevated HCT for the first 1 to 3 weeks post infection ($p < 0.05$). The 4 infected bulls which grew poorly also had a significantly higher infection intensity than the other infected bulls from Day 27 to Day 60 post-infection ($p < 0.05$). There was no pyrexia recorded in the infected group or control groups, instead there was a tendency for the infected group to have a lower rectal temperature from Day 5 to 70 post infection. The infected bulls walked on average 239 steps per day less than the control bulls, although this difference was not significant ($p = 0.35$).

Overall the study clearly showed, by controlling infection date and infectious dose, that a proportion of cattle infected with *T. orientalis* (Ikeda) have significantly decreased live weight gains.

1. Introduction

Since 2012 New Zealand has suffered a serious epidemic of bovine anaemia associated with *Theileria orientalis* (Ikeda) (Lawrence et al., 2016a). The epidemic has rapidly spread throughout most of the North Island and includes a lesser number of farms from the top and the west coast of the South Island (McFadden et al., 2016b). Environmental niche modelling has estimated that 64% of North Island cattle farms and 45% of New Zealand farms overall have a high suitability for *T. orientalis* transmission (Lawrence et al., 2016b). The current New Zealand epidemic is part of an ongoing East Asia, Australasia pandemic, with the disease reaching Australia in 2006 before New Zealand in 2012 (Izzo et al., 2010; Kamau et al., 2011; McFadden et al., 2013). The likely incursion pathway for the spread of infection between countries is the live importation of parasitaemic cattle into habitats populated by a genus of tick competent to transmit oriental theileriosis (Gebrekidan

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et al., 2017). Compared with New Zealand, which has only one, Australia has a greater number of tick genera that infest livestock. However, in both countries *H. longicornis* appears to be the most important *T. orientalis* vector (Hammer et al., 2016; Heath, 2016).

Sequence analysis of the major piroplasm surface protein (MPSP) gene has established that there are at least 11 distinct types of *T. orientalis* (Khukhuu et al., 2011; Watts et al., 2016) of which 4 types are currently recognised in New Zealand (Perera et al., 2015; Pulford et al., 2016a). Although only the Ikeda type is believed to be truly pathogenic, under certain circumstances the Chitose type has also been associated with severe clinical disease (Rawdon et al., 2006; McFadden et al., 2011).

The most serious effect of *T. orientalis* (Ikeda) infection is the associated anaemia which can be fatal. Even so, morbidity and mortality rates are still relatively low being < 1% (Shimizu et al., 1992; Vink et al., 2016). The anaemia is caused by extravascular haemolysis, although the exact pathogenesis is poorly understood (Lawrence et al., 2018a). Live weight effects have only rarely been reported or quantified (Kawamoto et al., 1991; Fukasawa et al., 2003). However, they can be inferred from the finding that "weight loss" was reported in 81/605 (13.4%) laboratory submissions for clinically infected cattle (Lawrence et al., 2017) and from 8/8 (100%) infected farms in an Australian study which reported the cows were suffering from "inappetence" (Izzo et al., 2010). When liveweight effects have been reported it is said that they occur in the convalescence period (Kawamoto et al., 1991), although what was meant by the convalescence period was not defined. Observations on both experimentally and naturally infected cattle agree that pyrexia is usually seen when piroplasms first appear in the peripheral circulation around 2 to 3 weeks post-infection (Uilenberg et al., 1985; Shimizu et al., 1990). A second temperature spike, at 4 to 5 weeks post-infection, was reported in splenectomised calves (Shimizu et al., 1990). In a review of 605 submission histories Lawrence et al. (2017) found only 72/605 (11.9%) mentioned pyrexia. Effects of *T. orientalis* (Ikeda) infection on activity have never been measured but logically there should be an effect of severe anaemia on activity. Again, this can be inferred, 162/605 (27%) submission histories (Lawrence et al., 2017) and 8/8 (100%) farms (Izzo et al., 2010) mentioned "lethargic" in the clinical history.

The objectives of this study were to infect a group of 2-year-old Friesian bulls with *T. orientalis* (Ikeda) and measure the effects on the haematocrit, weight, rectal temperature and activity, compared with a group of uninfected controls, over a follow-up period of 20 weeks.

2. Materials and methods

This was a randomised controlled experimental study of the effect of *T. orientalis* (Ikeda) infection on the live weight, haematocrit (HCT), temperature and activity of 2-year-old Friesian bulls. The experiment was conducted at Massey University's Tuapaka farm (latitude 41°24′S, longitude 175°36′E), 15 km east of Palmerston North, New Zealand, with approval from Massey University Animal Ethics Committee, Protocol 16/55.

From a group of 22 2-year-old Friesian bulls, 17 *T. orientalis* (Ikeda) PCR negative bulls were selected for the study. Five bulls were rejected for the following reasons: one bull had a marginal *Theileria orientalis* (Ikeda) PCR result, one was aggressive, one had small scrotal circumference and two had poor libido. Although the latter 2 reasons did not affect a bull's eligibility for the infection study, it did affect their eligibility for the fertility study which ran concurrently with the experimental infection study.

The bulls were fully vaccinated against leptospirosis and clostridial diseases (Ultravac 7in1, Zoetis New Zealand Ltd., Auckland, New Zealand) and for BVD (Ultravac BVD, Zoetis New Zealand Ltd., Auckland, New Zealand). They were well grown for their age and appeared healthy on visual examination. Initially the bulls were set stocked at pasture and fed at approximately maintenance to simulate the feed intake of a bull with a heavy mating workload. After 70 days, the length of a typical New Zealand mating period, the feed allowance was increased to ad libitum.

A random sample of 10 of the 17 selected bulls was inoculated with *T. orientalis* (Ikeda) using intravenous injection of 30 mL of infected blood. The infected blood was sourced from 2 acute cases of *Theileria*-associated bovine anaemia (TABA), that were confirmed positive for Ikeda via PCR, in a local dairy herd, which was experiencing a severe outbreak of TABA. The dairy farm was approximately 70 km from Tuapaka farm. From each affected cow 250 mL of blood was collected using a 450 mL blood collection bag with CPDA-1- anticoagulant (J-520, Jorgenson Laboratories Inc., Colorado, USA). Within 3 h of collection each of the 10 selected bulls was given 15 mL of blood from each of the two donor cows (total volume = 30 mL), by intravenous injection into the jugular vein. One bull experienced an anaphylactic reaction, suffering urticaria, muscle fasciculation and increased respiratory rates. This bull was successfully treated by intramuscular injection with 1 mg/kg mepyramine (Antimine, 50 mg/mL mepyramine maleate, Ethical Agents International Ltd., Auckland, New Zealand).

The day of injection was referred to as Day 1 of infection in the data analysis.

2.1. Haematocrit, temperature, weight and activity

Sampling of bulls started on the 18th August 2016, 29 days before *T. orientalis* (Ikeda) infection, and was completed by 21st February 2017, 138 days after *T. orientalis* (Ikeda) infection which was on the 16th September 2016. Commencing on the 19th September the bulls were blood sampled, temperature recorded and weighed 3 times a week on a Monday, Wednesday and Friday for 13 weeks before reducing the sampling frequency to once weekly, on a Wednesday, for a further 7 weeks. Prior to *T. orientalis* (Ikeda) infection the bulls had been blood sampled, rectal temperature recorded and weighed on 3 occasions at fortnightly intervals with the last sampling on 15th September, the day before *T. orientalis* (Ikeda) infection. The blood sampling, rectal temperature recording and weighing were all carried out sequentially, in that order, starting at 11.00 am on each sampling date.

Blood samples were collected into 10 mL EDTA tubes by venepuncture of the tail vein and after haematocrit measurement the blood was stored at −20 °C until molecular testing. The bulls' rectal temperature was recorded using a Digital Large Animal thermometer (Shoof International, Cambridge, New Zealand) and their body weights measured using a Tru-test EziWeigh7i weigh scales (Tru-Test Group, Auckland, New Zealand). The haematocrit was measured by reading micro-capillary tubes centrifuged for 5 min at 17,000*g*, using a Heraeus Pico 17 (Thermo Fisher Scientific, Langenselbold, Germany), with the average of two samples recorded for each bull. If the haematocrit for paired samples differed by > 2% then a repeat centrifugation and reading were performed.

Bull activity was measured using IceTags® ((IceRobotics, UK). The IceTag3D™ is a pedometer which uses three g-force accelerometers to measure animal activity in three dimensions (Kokin et al., 2014). These pedometers are waterproof and have sufficient memory to store the results of up to 60 days of measurements. They were strapped to the lateral side of each bull's left hind leg, just above the fetlock joint. Data download is wireless to a PC using an IceReader®, with data recorded on a per-second basis. For each animal, the time the bull spent lying and standing, the number of lying bouts, the number of steps and the motion index was computed using the dedicated IceTagAnalyser® software installed on the PC. The motion index measures the average magnitude of acceleration on each of the 3 axes (IceRobotics Ltd., Product Guide 2010). The "number of steps" data was initially aggregated per hour and then per day. Only 15 IceTags were available for the experiment so 6 control and 9 infected bulls were randomly selected and fitted with pedometers. The IceTags® were attached to the bulls from Days 9 to 60 and from Days 65 to 124, for a total of 111 days. They were not