The effects of *Theileria orientalis* Ikeda on bull fertility and libido

A dissertation presented in partial fulfilment of the requirements for
the degree of

Master of Science

At Massey University, Palmerston North

New Zealand

Michaela Jane Gibson

2017
Abstract

*Theileria orientalis* is a blood-borne parasite that is prevalent in New Zealand and other countries. The recent emergence (2012) of Ikeda-type *orientalis* has resulted in an epidemic of bovine anaemia in both dairy and beef cattle herds. The disease is spread by *Haemaphylis longicornis* which is prevalent in the majority of the North Island.

The Ikeda type has been found to be more pathogenic than previously discovered types such as Chitose and Buffeli. Little is known about how Ikeda-type affects the reproductive performance of bulls. The aim of this experiment was to examine the effects of *Theileria* Ikeda on the fertility and libido of bulls. A group of 17 bulls were used in the experiment with 10 being infused with *Theileria* Ikeda-infected blood from two donor cows and the remaining 7 bulls used as controls. All 10 of the treatment bulls were successfully infected with *Theileria* and became clinically anaemic (Haematocrit below 24) between days 47 and 84 post transfusion. Semen and libido was tested every 2 weeks throughout the experiment. There was no observed change in wave motion score of semen between infected (7.51 ± 0.18) and control (7.08 ± 0.35) treatment groups (P=0.2935) along with no change in forward motion between infected (7.82 ± 0.16) and control (7.64± 0.2610) treatment groups (P=0.5579). The percentage of normal sperm (P=0.0032) was lower in the infected bulls (91.9 ± 0.05) compared to the control group (94.25 ± 0.06) although the density of sperm in an ejaculate (P=0.0044) was higher in infected bulls (1.45x10^10 ± 6.88x10^6 sperm per mL) compared to control bulls (1.14x10^10 ± 9.82x10^6 sperm per ml). Time to first mount (P=0.7374) and gap between first and second mount (P=0.2204) was not significantly different between infected and control groups. The number of mounts was similar between infected (2.33 ± 0.28) and control (2.36 ± 0.17) treatment groups (P= 0.9269) and there was no interaction with time (P=0.2221). However, there was a significant effect of treatment on order of service with infected bulls coming in to the yard later in the herd on day 55 and was statistically significant (P=0.02). In conclusion, changes in fertility occurred in infected bulls but were not drastic enough to indicate a decrease in overall fertility. The only measure of libido affected was order and it is unknown how this would affect pregnancy rates in a herd situation.
Acknowledgments

I would firstly like to thank my supervisors Dr Rebecca Hickson and Dr Bill Pomroy for the opportunity to be a part of this research project along with their support and guidance throughout my time as a Masters student.

Secondly, I would like to thank Kevin Lawrence, Kristene Gedye, Anne Tunnicliffe, Barbara Adlington, Stefan Smith from IVABS, Jono Brophy from Tuapaka farm and Robyn Howe, Guy Haynes, Courtney Moffat from Tararua Breeding Centre for their help and expertise during the experiment and completing my thesis.

Thirdly I would like to thank Massey University and Beef + Lamb New Zealand for funding the experiment, and the Johannes August Anderson postgraduate scholarship, Taranaki Dairy Farmers Conference Scholarship and the Hurley Fraser Postgraduate Scholarship for their generous financial support in completing my masters.

And lastly I would like to thank my friends and family especially my parents for supporting and believing in me through the highs and lows of my university studies.
# Table of contents

Abstract ....................................................................................................................................... i

Acknowledgments ...................................................................................................................... ii

Table of contents ...................................................................................................................... iii

List of tables .............................................................................................................................. vi

List of Figures ........................................................................................................................... vii

List of abbreviations .................................................................................................................. ix

1.0 Introduction .................................................................................................................... 1

2.0 Literature Review ................................................................................................................. 2

  2.1 *Theileria* ........................................................................................................................... 2

    2.1.1 History ........................................................................................................................ 2

    2.1.2 Mechanism of infection ............................................................................................. 4

    2.1.3 Distribution ................................................................................................................ 8

    2.1.4 Pathophysiology and Immunological effects leading to anaemia .......................... 11

    2.1.5 Clinical signs ............................................................................................................. 12

    2.1.6 Diagnosis of infection .............................................................................................. 12

    2.1.7 Effects on production .............................................................................................. 13

    2.1.8 Treatment ................................................................................................................ 14

    2.1.9 Prevention ............................................................................................................... 15

  2.2 Libido .............................................................................................................................. 17

    2.2.1 Importance .............................................................................................................. 17

    2.2.2 Social hierarchy ...................................................................................................... 17

    2.2.3 Soundness ................................................................................................................ 19

    2.2.4 Physiology ................................................................................................................ 19

    2.2.5 Measuring libido ...................................................................................................... 21

  2.3 Fertility ........................................................................................................................... 23

    2.3.1 Introduction ............................................................................................................. 23

    2.3.2 Spermatogenesis ..................................................................................................... 23

    2.3.3 Hormonal control .................................................................................................... 24

    2.3.4 Spermatogenesis ..................................................................................................... 24

    2.3.5 Sperm differentiation .............................................................................................. 25
2.3.6 Disease affecting spermatogenesis ................................................................. 25
2.3.8 Hormonal level ................................................................................................. 27
2.3.9 Factors affecting spermatogenesis ................................................................. 30

3.0 Materials and methods ....................................................................................... 35
3.1 Animals/Management ......................................................................................... 35
3.1.1 Selection ........................................................................................................... 35
3.1.2 Feeding and management ............................................................................. 35
3.2 Treatments ............................................................................................................ 36
3.2.1 Infection .......................................................................................................... 36
3.3 Measurements ...................................................................................................... 36
3.3.1 Theileria .......................................................................................................... 36
3.3.2 Libido ............................................................................................................. 36
3.3.3 Semen evaluation ......................................................................................... 37
3.3.4 Lab methods ................................................................................................... 39
3.4 Data handling ...................................................................................................... 41
3.5 Statistical analysis .............................................................................................. 41

4.0 Results .................................................................................................................. 43
4.1 Blood .................................................................................................................... 43
4.1.1 PCR .................................................................................................................. 43
4.1.2 HCT ................................................................................................................ 45
4.2 Weight .................................................................................................................. 45
4.3 Temperature ........................................................................................................ 47
4.4 Clinical Pathology ............................................................................................... 48
4.5 Libido .................................................................................................................. 52
4.5.1 Time to first mount ....................................................................................... 52
4.5.2 Gap from first to second mount ................................................................. 55
4.5.3 Number of mounts ....................................................................................... 58
4.5.4 Order ............................................................................................................. 59
4.6 Fertility ................................................................................................................ 60

5.0 Discussion ............................................................................................................ 64
5.1 Future work ......................................................................................................... 68
5.2 Limitations .......................................................................................................... 69
6.0 Conclusion........................................................................................................................................70
6.1 Main findings....................................................................................................................................70
6.2 Implications ....................................................................................................................................70
7.0 References ......................................................................................................................................72
8.0 Appendices......................................................................................................................................80
8.1 Appendix 1 Yard plan for Tuapaka farm used for libido testing .................................................80
8.2 Appendix 2 Semen abnormalities .................................................................................................81
8.4 Appendix 4 SAS code for Libido ..................................................................................................82
8.5 Appendix 5 SAS Code for Semen traits .......................................................................................89
8.6 Appendix 6 R code for service order, Haematocrit, PCR and order ...........................................93
8.7 Appendix 7 Standard operating procedure for semen analysis ..................................................98
8.8 Appendix 8- Standard operating procedure for quantification of Theileria Ikeda .....................102
8.9 Appendix 9- Standard operating procedure for libido testing of bulls ......................................106
List of tables

Table 3.1: Wave motion score chart based on microscope observations at 200x magnification (Chenoweth, 2015) .................................................................................................................. 38

Table 3.2 Polymerase chain reaction sequences for primers and probe ................................................. 40

Table 4.1 Total weight gain and average daily gain of infected and control bulls ......................... 46

Table 4.2 Haematology results for uninfected and infected samples taken on 35 days post infection.............................................................................................................................................. 49

Table 4.3 Haematology results for infected and control bulls on Day 66 with P-values. Values with statistically significant values are highlighted in bold .................................................................................. 50

Table 4.4 Additional haematology results for infected and control bulls on Day 66 with P-values. Values with statistically significant values are highlighted in bold ......................................................................... 51

Table 4.5 Summary for test of equality over strata for each day post infection for time to first mount. ........................................................................................................................................... 53

Table 4.6 Summary of test of equality over strata for each day post-infection for gap between first and second mount. .................................................................................................................. 56

Table 4.7 Mean number of mounts for each treatment group, standard error and P-value .... 58

Table 4.8 Mean values for wave motion, forward motion, density and morphology with standard error and p-values ........................................................................................................................................ 61
List of Figures

Figure 2.1: Number of herds with cases of anaemia associated to Ikeda type diagnosed between the 1st of August 2012 and the 30th of March 2014 as well as the spatial distribution of the case herds in the North Island of New Zealand. Period 1 (red) was from 30th August 2012 to 28th of February 2013, period 2 (blue) was from the 1st of March 2013 until the 31st of July 2013 and period 3 (green) was from 1st August 2013 until the 4th of March 2014 (Lawrence et al., 2016b)............................................................................................................4

Figure 2.2: A generalized lifecycle of Theileria parva (Mans et al., 2015).................................6

Figure 2.3: The distribution of H. longicornis in New Zealand. Black regions are where the tick is the most common and the grey regions correspond with areas where the tick is less common (Heath, 2016)..............................................................................................................8

Figure 2.4: Hard tick lifecycle with the egg, larve, nymph and adult stages shown. The Figure also shows the stages that require a host (Kaye, 1974).................................................................9

Figure 2.5: The seasonal pattern of H. Longicornis activity under optimum conditions. Where (a) is overwintering eggs, larvae, nymphs and adults, (b) is the nymphal peak caused by unfed nymphs from winter, (c) early adult activity from engorged nymphs that were dormant over winter, (d) is early larval activity from unfed larvae and eggs that have overwintered: these cause the rise in active nymphs in late spring to early summer, (e) is the main adult activity period caused by the nymphal peak (b), this causes the main larval activity period (F), and (g) is the smaller peak in nymphal activity from late larval feeding and moulting in mid-summer which causes the nymphal peak (c). Since nymphs and adults are the only stages that spread Theileria. Therefore, peak infection times would be in peaks (e) and (f) and to a lesser extent in peak (c) and (d) (Heath, 2016).........................................................10

Figure 2.6 The process of spermatogenesis along with heat sensitive stages (Ivell, 2007).....24

Figure 2.7 Mean cortisol concentrations between pre-infection and infection in animals infected with Trypanosoma congoense (Abebe et al., 1993)..............................................................................28

Figure 2.8 Histological image of a bull with normal seminiferous tubules filled with spermatogenic cells (top picture) and an image of the epididymis showing normally formed tubules with sperm reserves (Adamu et al., 2007).........................................................................................32

Figure 2.9: A histological image of the testis of a bull infected with Trypanosoma vivax. The top image shows severe degeneration of germinal epithelial cells in seminiferous tubes(s). The bottom image shows the epididymis of the same infected bull with the absence of epididymal sperm reserves (Adamu et al., 2007).................................................................................................................33

Figure 4.1 Loess smoothed regression line, span=0.2, count of organisms per mL against days from infection for control (red line) and infected bulls (black lines) with 95% confidence (grey area). The horizontal dotted line is the cut point of 1 fg/μl. .........................................................44
Figure 4.2 Loess smoothed regression line, span=0.2, of haematocrit against days from infection for control (red line) and infected bulls (black line) with 95% confidence (grey area). The vertical dotted line is the infection day and the blue box from Day 47 to Day 80 are the days when the HCT of the infected bulls was significantly lower (adjusted P<0.05) than the control bulls. ..........................................................................................................................45

Figure 4.3 Loess smoothed regression line, span=0.4, of body weight against days from infection for control (red line) and infected bulls (black line) with 95% confidence (grey area). The vertical dotted line is the infection day..........................................................46

Figure 4.4 Loess smoothed regression line, span=0.4, of rectal temperature against days from infection for control (red line) and infected bulls (black line) with 95% confidence (grey area). The vertical dotted line is the infection day. ........................................................................47

Figure 4.5 The median deviation time to first mount relative to pre-infection measures of time to first mount for bulls infected with *Theileria* infected ( -, n=10) or uninfected control bulls ( --, n=7) at fortnightly libido tests after infection...............................52

Figure 4.6- Product-limit survival estimates for each libido testing day post-infection for time to first mount...............................................................................................................................................54

Figure 4.7 The median deviation time between gap from first and second mount relative to pre-infection measures of gap for bulls infected with *Theileria* infected ( -, n=10) or uninfected control bulls ( --, n=7) at fortnightly libido tests after infection...............................55

Figure 4.8 Product-limit survival estimates for each libido testing day post-infection for time to first mount...............................................................................................................................................57

Figure 4.9 The average number of mounts for bulls infected with *Theileria* ( -, n=10) or uninfected control bulls ( --, n=7) at fortnightly libido tests after infection. There was no significant effect of treatment (P=0.9269), day (P=0.2106), on the number of mounts. There was no interaction (P=0.2221) between treatment and day. .................................................................58

Figure 4.10 Plot of average service number, with error bars, for infected, ---, and control, - , bulls against days from infection. Blue line represents the expectation for random order of bulls.............................................................................................................................................59

Figure 4.11 Average wave motion for bulls infected with *Theileria* infected ( -, n=10) or uninfected control bulls ( --, n=7) at fortnightly libido tests after infection. ...............................................................61

Figure 4.12 Forward motion for bulls infected with *Theileria* infected ( -, n=10) or uninfected control bulls ( --, n=7) at fortnightly libido tests after infection.................................................................................................62

Figure 4.13 Average density for bulls infected with *Theileria* infected ( -, n=10) or uninfected control bulls ( --, n=7) at fortnightly libido tests after infection.................................................................................................62

Figure 4.14 Average percentage of normal for semen samples collected from *Theileria* infected ( -, n=10) or uninfected control bulls ( --, n=7).................................................................................................................................................63
List of abbreviations

TABA- Theileria associated bovine anaemia

MPSP- Major piroplasm surface protein

qPCR- Quantitative polymerase chain reaction

FANI card- Field anaemia nearest indicator card

HCT- Haematocrit

LH- Luteinizing hormone

GnRH- Gonadotrophin releasing hormone

FSH- Follicle stimulating hormone

ABP- Androgen binding hormone

HPT- Hypothalamo-pituitary- testicular axis

ASA- Antisperm antibodies

DNA- Deoxyribonucleic acid