The application of immunological, molecular and epidemiological approaches to the study of Neosporosis in cattle

by

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Certificate of authorship and originality

I certify that the work in this thesis has not been previously submitted for a degree, nor has it been submitted as a part of requirements for a degree except as fully acknowledged in the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signed

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Publications arising from this thesis

Articles in refereed journals

CA Hall, MP Reichel, JT Ellis (2005) *Neospora* abortions in dairy cattle: diagnosis, mode of transmission and control. Veterinary Parasitology 128, 213-241.
(This paper was the 11th most downloaded article from Veterinary Parasitology in the 2nd quarter of 2005. It also has a citation index of 3 (in only 3/4 of a year)).

CA Hall, MP Reichel, JT Ellis (2005) Performance characteristics of two enzyme-linked immunosorbent assays for the detection of antibodies to *Neospora caninum* in the serum of cattle. Accepted for publication in Veterinary Parasitology, March 2006.

CA Hall, MP Reichel, JT Ellis (2005) Validation and performance characteristics of an enzyme-linked immunosorbent assay for the detection of antibodies to *Neospora caninum* in the milk of cattle and subsequent application to determine prevalence of infection in NSW dairy cattle. Submitted to Veterinary Parasitology, December 2005.

Magazine article

CA Hall, MP Reichel, JT Ellis (2005) Control of *N. caninum* infection: a case study using test and cull and the value of serology. The Australian Cattle Veterinarian 35, 14-16.

Conference proceedings

C Hall, MP Reichel and JT Ellis (2003). Evidence of vertical transmission of *Neospora caninum* on a dairy farm in NSW. The 45th Annual Scientific Meeting of the Australian Society for Parasitology, 7-10 July, Darwin, Australia: abstract C24.

C Hall, MP Reichel, JT Ellis (2004) Control of *Neospora caninum* infection in a NSW dairy herd. Proceedings of the 1st Australian Dairy Science Symposium, 26-27 February, Shepparton, Australia: pg 42, poster 7.

C Hall, MP Reichel, JT Ellis (2004) Vertical transmission of *Neospora caninum* in a NSW dairy herd. Proceedings of the 1st Australian Dairy Science Symposium, 26-27 February, Shepparton, Australia: abstract pg 16.

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List of Abbreviations

AI	artificial insemination
ANGIS	Australian National Genomic Information Service
BHV	bovine herpes virus
bp	base pair
BVD	Bovine Viral Diarrhoea
BVDV	Bovine Viral Diarrhoea virus
CMDT	computational methods for diagnostic tests
d	days
DIM	days in milk
ELISA	enzyme linked immunosorbent assay
GHRL	Gore Hill Research Laboratories, Gore Hill, NSW, Australia
h	hours
IBR	Infectious Bovine Rhinotracheitis
IFAT	indirect fluorescent antibody test
IFN-γ	gamma interferon
IHC	immunohistochemistry
IL	interleukin
IP	Institut Pourquier
ITS	internal transcribed spacer
min	minute
mon	months
MQ	MilliQ
NCBI	National Centre for Biotechnology Information
NSW	New South Wales, (Australia)
OD	optical density
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PI	persistently infected
rDNA	ribosomal DNA
RT	room temperature

SCC	somatic cell count
SCID	severe combined immunodeficient
Se	sensitivity
Sp	specificity
TG-ROC	two graph-receiver operating characteristics
TMB	tetramethylbendizine
UK	United Kingdom
USA	United States of America

Abstract

A prospective study was undertaken on a 260-head dairy herd in NSW, Australia to determine the modes of transmission, prevalence, effect on abortion and reproduction parameters and the impact on milk production of *Neospora caninum*. A test-and-cull method of control was also evaluated. The possible infectivity of milk from *N. caninum* infected cows was also investigated using a mouse model. As ELISAs form an important part of diagnosing *N. caninum* infection, studies were undertaken to evaluate two serum and one milk ELISA. Analysis was performed for each of the ELISAs to determine cut-off thresholds, Se and Sp. The milk ELISA was subsequently used to determine the overall prevalence of *N. caninum* in NSW dairy cows.

In the prospective study, 11.4% of the herd's cattle were seropositive to *N. caninum* by ELISA and the dominant route of transmission was vertical as the majority of the infected cattle were related and only a few seropositive cattle were born from seronegative dams (*i.e.* reflecting postnatal transmission). As 90% of offspring born from seropositive cows were also seropositive this suggests a high vertical transmission rate. *Neospora caninum* was found to be a major cause of abortion as cows seropositive to *N. caninum* had a 13-fold higher risk of abortion then seronegative cattle. Early foetal loss was also predicted to be associated with *N. caninum* infection as seropositive cows required a significantly greater number of inseminations and took longer to conceive than seronegative cattle. BVDV and IBR alone were not associated with causing abortion in this herd. This is the first report of an effective control strategy for *N. caninum* by either culling seropositives or not breeding from seropositive cows. This method was effective in reducing the number of infected cattle and was feasible due to the low prevalence of *N. caninum* on the farm and thus did not place too high a financial burden on the farmer. *Neospora caninum* DNA was also detected by PCR on milk samples from seropositive cows. This is the first Australian report demonstrating *N. caninum* DNA in milk. Of the serum ELISA evaluated, one was determined to have high Se and Sp at the cut-off recommended by the manufacturer while the other required modification of the cut-off value to gain the same high Se and Sp. After choosing the most suitable milk dilution, the milk ELISA was determined to have high

Se and Sp of 97%. Using this ELISA the prevalence of *N. caninum* in NSW dairy cattle was determined to be 21.1%.

Neospora caninum was found to be a significant cause of abortion and also foetal loss in the study herd and was vertically transmitted efficiently. Control efforts using a test-and–cull approach were successful without placing economic hardship on the farmer. The evaluation of several ELISAs was useful as these can be used in diagnosing infection. In particularly the milk ELISA will now make sampling easier so enabling whole herd sampling by the farmer. This ELISA may be of particular use in test-and cull programs or in epidemiological studies. The concept of detecting *N. caninum* DNA in milk could be of great importance to the dairy industry as it suggests a new route of infection. Although milk extracts from seropositive cows were not infective to SCID mice in this study this should be investigated further.