Some aspects of the epidemiology of neosporosis in sheep in New Zealand

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Sharifah Salmah SYED-HUSSAIN

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khas buat mak dan abah.

"Never say quit till u die....."

Abstract

Recent reports from New Zealand indicate *Neospora caninum* may have a role in causing reproductive problems in sheep. However, knowledge about the epidemiology of neosporosis in sheep in New Zealand is limited. Thus, the research presented in this thesis was undertaken to further understand the mode of transmission, seroprevalence, diagnosis and treatment of *N. caninum* in sheep in New Zealand.

The initial study investigated venereal and vertical transmission. The results suggested that although *N. caninum* DNA can be found in the semen of experimentally infected rams (n=16), the transmission of *N. caninum* to ewes (n=16) via natural mating is unlikely. In a two year study, ewes inoculated prior to mating (n=25 in Year 1; n=7 in Year 2) did not have congenitally infected lambs that year (n=0/44) but did in Year 2 (n=7/11). Ewes re-inoculated on Day 120 of gestation in Year 2 (n=9) had congenitally infected lambs (n=12/12) with more severe lesions than those not re-inoculated (n=2/11) indicating that the initial inoculation did not induce protection. Ewes inoculated for the first time on Day 120 of gestation (n=12) gave birth to lambs (n=10) that were all congenitally infected. Treatment of these congenitally infected newborn lambs (n=11) with toltrazuril (20mg/kg) on Day 1, 7, 14 and 21 was not effective as determined by serology, histopathology and qPCR.

An avidity ELISA assay was able to differentiate between recently and chronically infected sheep. A longitudinal study with serology on 3 farms where *N. caninum* infected sheep were previously identified, found an overall seroprevalence of 0.8% (n=7/880) for *N.*

caninum antibodies. The low seroprevalence observed across selected farms did not allow a meaningful interpretation to be made about the role of neosporosis on these farms.

A consistent observation was the value of using multiple diagnostic tests to detect the presence of *Neospora* rather than relying exclusively on any one of them. Observation of typical lesions was generally more rewarding then the detection of *Neospora* DNA. Overall, further work is required to fully determine if *N. caninum* is causing reproductive problems in sheep in New Zealand.

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List of Abbreviations

AI	artificial insemination
ANOVA	analysis of variance
AV	artificial vagina
BLAST	basic local alignment search tool
cELISA	competitive ELISA
CI	confidence interval
CNS	central nervous system
DAT	direct agglutination test
DNA	deoxyribonucleic acid
dNTPs	2'-deoxynucleotide 5'-triphospate
ds	double strand
ELISA	enzyme-linked immunosorbent assay
FBS	foetal bovine serum
G	gravity
H&E	haematoxylin and eosin
IDA	immunodominant antigen
IFAT	indirect fluorescent antibody test
iELISA	indirect ELISA
IFN	interferon
IFN-γ	gamma interferon
Ig	immunoglobulin
IgG	immunoglobulin G
IgM	immunoglobulin M
IH	in-house
IHC	immunohistochemistry
IL	interleukin
ISCOM	immunostimulating complex
IVABS	Institute of Veterinary, Animal and Biomedical Sciences, Massey University
i/v	intravenously
kDa	kilodalton
LAT	latex agglutination test
rDNA	ribosomal DNA

M	molar
MEM	minimum essential medium
NcNZ	*N. caninum* New Zealand
NcSAG1	*N. caninum* surface antigen 1
NcSRS2	*N. caninum* surface antigen-1 related sequence 2
NK	natural killer
O	oocysts
OD	optical density
PBS	phosphate buffer saline
PCR	polymerase chain reaction
qPCR	quantitative or real time PCR
r	ribosomal
RNA	ribonucleic acid
S	Svedberg units
s	seconds
SAG	surface antigen
SDS PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
S/P	sample to positive ratio
SC	sub cutaneous
ss	single strand
t	tachyzoites
TPI	transplacental infection

Thesis structure and format

This thesis is presented as a series of chapters that are inter-related, thus there are some repetitive elements especially in the Materials and Methods section. Four of the chapters have been published (Chapters 2, 3, 4 and 5) in peer reviewed journals. They are included in the thesis in the form they are presented for publication except that sections have been renumbered and references to appendices have been included. All references for each chapter have been collated at the end of the thesis in the Bibliography section.

Chapter One: ***Neospora caninum*** **– General introduction and literature review** introduces a brief summary of the objectives of the thesis. It includes a review on various aspects of neosporosis especially in cattle and sheep.

Chapter Two: **Detection of *Neospora caninum* DNA in the semen of experimentally infected rams with no evidence of horizontal transmission in ewes** has been published in the journal Veterinary Parasitology (Syed-Hussain, S.S., Howe, L., Pomroy, W.E., West, D.M., Smith, S.L., Williamson, N.B., 2013, 197, 534-542) which suggested that venereal transmission of *N. caninum* is not a possible route of transmission in sheep although the parasite DNA was detected in the semen of these experimentally infected rams.

Chapter Three: **Adaptation of a commercial ELISA to determine the IgG avidity in sheep experimentally and naturally infected with *Neospora caninum*** has been published in the journal Veterinary Parasitology (Syed-Hussain, S.S., Howe, L., Pomroy, W.E., West, D.M., Smith, S.L., Williamson, N.B., 2014, 203, 21-28) and describes the use of a commercially available ELISA test kit that has been adapted as an IgG avidity assay to differentiate between recently and chronically infected sheep.

Chapter Four: **Vertical transmission of *Neopsora caninum* in experimentally infected sheep** has been published in the journal Veterinary Parasitology (S.S. Syed-Hussain, L. Howe, W.E. Pomroy, D.M. West, M. Hardcastle, and N.B. Williamson., 2015) and describes the importance of vertical transmission as a mode of transmission of neosporosis in sheep. However how this reflects in a natural setting is yet to be known. This study was run concurrently as a part of another study which is described in Chapter Five.

Chapter Five: **A study on the use of toltrazuril to eliminate *Neospora caninum* in congenitally infected lambs born from experimentally infected ewes** has been published in the journal Veterinary Parasitology (S.S. Syed-Hussain, L. Howe, W.E. Pomroy, D.M. West, M. Hardcastle, and N.B. Williamson., 2015) and describes the results of the use of toltrazuril as a mode of treatment for neosporosis in congenitally infected newborn lambs.

Chapter Six: **A longitudinal investigation of *Neospora caninum* serodynamics and seroprevalence in naturally-infected pregnant ewes** describes the serological status of sheep on farms with a previous history of *N. caninum* infection over pregnancy.

Chapter Seven: **General discussion** summarises all the information observed from the studies above which also includes the implications of the findings as well as suggestions for future research work.

Bibliography: To minimise repetition, all references are listed at the end of the thesis.

All experiments conducted in this thesis were approved by the Massey University Animal Ethics Committee.