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**STUDIES ON *NEOSPORA CANINUM* AND NEOSPOROSIS IN NEW
ZEALAND CATTLE**

A thesis submitted in partial fulfilment of the requirements for the degree of

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In

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General Abstract

The objective of this research was to investigate neosporosis in New Zealand cattle using experimental and molecular tools. The research also aimed to isolate and characterise an indigenous New Zealand isolate of *Neospora caninum*. A series of discrete projects were conducted to achieve the set objectives. A pilot study was first conducted and a wild-type strain of non-cytopathic bovine viral diarrhoea virus (BVDV) type 1 virus was isolated *in vitro* from a persistently infected heifer. The isolate was used to challenge heifers and the effect of experimental BVDV infection on heifers naturally infected with *N. caninum* was investigated. Results showed that heifers that were both BVDV and *N. caninum* seropositive produced 44.4% (8/18) dam-calf pairs that were both BVDV and *N. caninum* seropositive. Serologically, 66.7% (12/18) dam-calf pairs were seropositive to *N. caninum* antibodies, while 80% (8/10) dam-calf pairs were BVDV seropositive. A Polymerase Chain Reaction (PCR) study was also conducted and *N. caninum* DNA was detected in the blood of naturally infected aborting and pregnant heifers. Real-time quantification of *N. caninum* DNA in the blood of infected heifers showed a decrease of *N. caninum* DNA after abortion in the aborting group and an increase through gestation in the pregnant group. A study of antigenicity recognised 7 immunodominant (~18, ~25, ~33, ~35-36, ~45-46, ~47 and 60-62 kDa) and 5 minor antigens of *N. caninum* by cow sera. Three isolates of *N. caninum* (NcNZ 1, NcNZ 2 and NcNZ 3) were isolated from the brains of an infected cow, her calf and a stillborn calf. These isolates were confirmed as *N. caninum* by PCR, immunofluorescence antibody test and immunohistochemistry and were pathogenic to BALB/c mice.

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Dedication

This work is dedicated to my mother-in-law, Madam Iheanahuru Enyo Okeoma, who passed on as this work was being compiled and father-in-law, Mazi Obierezie Okeoma, who also did not live to see the award of this degree. To my parents, Mr. Pius and Mrs. Felicia Egbujiobi, your hard work and sacrifices will always be appreciated.

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Abbreviations

aa	Amino acid
BELU	Bovine embryonic lung
BLAST	Basic local alignment search tool
Bp	Base pairs
BSA	Bovine serum albumin
BVDV	Bovine viral diarrhoea virus
CO ₂	Carbon dioxide
cp	Cytopathic
DAB	Diaminobenzidine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotides triphosphates
ELISA	Enzyme linked immunosorbent assay
ERMA	Environmental Risk Management Authority
FBS	Foetal bovine serum
FITC	Fluorescein isothiocyanate
GMO	Genetically modified organism.
IFAT	Imunofluorescent antibody test
IFN	Interferon
IgE	Immunoglobulin E
IgG	Imunoglobulin gamma
IHC	Immunohistochemistry
IGS	Intergenic spacer
IL	Interleukin
ITS 1	Internal transcribed spacer 1
kDa	Kilodalton
MEM	Minimum essential medium
MgCl ₂	Magnesium chloride
NaCl	Sodium chloride
NCP	Non-cytopathic
NcNz	<i>Neospora caninum</i> New Zealand
NeoF	<i>Neospora</i> forward

NeoR	<i>Neospora</i> reverse
OD	Optical density
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PV	Parasitophorous vacuole
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SAS	Statistical analysis system
SDS-PAGE	Sodium dodecyl sulphate polyacrilamide gel electrophoresis
Se	Sensitivity
Sp	Specificity
S/P ratio	Sample/positive ratio
TBE	Tris-Borate-EDTA
TEM	Transmission electron microscopy
<i>T. gondii</i>	<i>Toxoplasma gondii</i>
Th	T helper
TNF	Tumour necrosis factor
UNG	Uracil N-glycosylase
UTR	Untranslated region
UV	Ultra violet
VMRD	Veterinary medical research and development
VNT	Virus neutralisation Test