EPIDEMIOLOGY OF INFECTION WITH *LEPTOSPIRA* SPECIES IN LIVESTOCK IN PAPUA NEW GUINEA

Thesis submitted by

Peter Meiwan Wai'in
BSc (UPNG), MSc (Virology) (University of London)

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Murdoch University

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Declaration

I declare this thesis is my own account of my research and contains as its main content work which has not been previously submitted for a degree at any tertiary education institution.

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Peter Meiwan WAI'IN

Abstract

The role of infection with *Leptospira* as a cause of infertility in Papua New Guinea (PNG) has not been confirmed, mainly because of the lack of robust and simple diagnostic tests in PNG. The aims of this study were to determine the seroprevalence and distribution of infection in livestock in PNG and to develop and validate a diagnostic test for use in PNG that was sufficiently accurate and reliable for confident interpretation of the results. The nested and real-time PCRs were assessed for use as diagnostic tools.

The first survey was conducted on 3 commercial, 3 smallholder cattle farms and 4 abattoirs in March 2004 in PNG. Each herd was stratified into 3 age groups (< 2, 2-5 and >5 years), and sera from 1379 animals were sampled in Lae and Kimbe. In addition, 73 kidneys were collected from cattle at the abattoir and aseptically processed for culture. Two hundred and eighty three sera were collected from pigs killed at the abattoirs and 79 pig kidneys were collected and cultured. All sera were tested using the microscopic agglutination test (MAT). The dominant serovar infecting the cattle in PNG was Hardjo with a seroprevalence of 53.7%. The prevalence of serovar Hardjo in the six farms and the abattoir was significantly higher than serovars Tarassovi and Pomona ($P < 0.05$). All pig sera were negative for *Leptospira*. Leptospires were isolated by culture and the isolates were typed and identified as *L. borgpetersenii* serovar Hardjo. Cattle are a recognized reservoir for serovar Hardjo and may have a role in transmission to humans.

The second survey was conducted in June 2006 to determine if cattle from smallholder farmers, village pigs and dogs in the Markham Valley in Lae, PNG were infected with *Leptospira*. In addition, pigs from a commercial piggery and horses from commercial and smallholder farms were also sampled. A total of 69 pig sera, 22 dog sera, 15 horse sera and 111 cattle sera were collected. The results showed that 1 dog and 1 pig were seropositive with serovar Canicola. Of the 111 cattle sampled, 21 were seropositive for Hardjo. It was concluded that the seroprevalence with serovar Hardjo in these cattle was significantly lower than cattle from commercial properties. Smallholder cattle may therefore not be a major source of Hardjo infection for animals on commercial farms and pigs do not appear to be infected with *Leptospira*.

The Ab-ELISAs were constructed using one crude preparations of *L. interrogans* serovar Pomona and 2 different crude preparation of *L. biflexa* serovar Patoc. The three antigen preparations were evaluated using 21 MAT-positive and 96 MAT-negative pig sera to determine which antigen preparation was suitable for use in an Ab–ELISA. The selected antigen preparation (L1) was validated in the test using serum from 2 cattle and 1 pig population that were seropositive for *Leptospira*. A sub-population of seronegative cattle and pigs were also used. The Ab-ELISA was used to test 1,465 bovine sera from 8 cattle populations and the results were compared with the MAT using a Bayesian framework, to obtain an unbiased estimate of the accuracy of the tests. The ELISA had high sensitivity and specificity. Results from the Bayesian analysis showed that the sensitivity and specificity estimates for the Ab-ELISA were high compared to the MAT. Based on the test accuracy and its performance the Ab-ELISA using the L1 antigen described in this study is suitable for use in countries like PNG where the MAT is difficult to perform.

Samples of kidneys from livestock in PNG were tested using culture and a PCR-based assay to detect *Leptospira* species. A total of 72 samples of kidney were collected from cattle and a total of 74 samples were collected from pigs slaughtered in Lae and Port Moresby. A second study was designed to assess the use of a real-time PCR for detecting leptospiral DNA in urine from cattle. One hundred and ninety-three urine samples were collected from a beef cattle farm in WA. Whole genomic DNA from kidney samples was extracted from each kidney using the QIAamp DNA Mini kit (Qiagen). Heat lysis was used to extract genomic DNA from clear urine samples and the QIAamp Mini Kit was used for urine that was contaminated with faeces. The PCR-based test was able to detect a higher number of *Leptospira*-positive kidneys compared to culture in EMJH medium. Results of testing DNA extracted from urine using the real-time PCR showed that this test is sensitive and able to detect cattle infected with pathogenic leptospires.

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This thesis is dedicated to my dear dad, David Bomboman Wai'in who passed away in March 2006.

"Though you left me, you're never gone"

Abreviations

<	less than
>	greater than
≥	greater than or equal to
%	percent
µ (pref1x)	micro (10^{-6})
MAT	microscopic agglutination test
EMJH	Ellinghausen-McCullough-Johnson-Harris medium
^{o}C	degrees Celsius
Ab-ELISA	antibody-detection enzyme-linked immunosorbent assay
ABTS	2,2'-azino-di-(3-ethylbenzylhiazoline-6-sulfonate)
AUC	area under the curve
CI	confidence interval
C_T	threshold cycle
$d_{(0)}$	cut-off value calculated from the TGROC computer program that gives equal test sensitivity and specificity
DMSO	dimethyl sulfoxide
EDTA	ethylenediamine-tetraacetic acid, tri potassium salt
ELISA	enzyme-linked-immunosorbent assay
FAO	Food and Agriculture Organisation
et al.	and others
g	unit of gravitational field
HRP	horseradish peroxidase
IgG	immunoglobulin G
IgM	immunoglobulin M
IR	intermediate range for the cut-off value for the ELISA calculated using the TGROC computer program
kb	kilo base pairs (a unit of measurement of DNA)
kHz	kilohertz (10^3 cycles per second)
L	litre
LDC	Livestock Development Corporation
LR	likelihood ratio
m	metre

M	molar concentration
min	minute
mm	millimetre
ml	millilitre
NaCl	sodium chloride
NHSS	Numundo half stand system
NPOL	New Britain palm oil limited
NPV	negative predictive value
WHO	World Health Organisation
sq km	square kilometre
OIE	Office International Des Epizooties
OD	optical density
ORF	open reading frame
P	probability of an event due to chance alone
PBS	phosphate buffered saline
pH	minus log of the hydrogen ion concentration
PCR	polymerase chain reaction
PNG	Papua New Guinea
PPV	positive predictive value
ROC	receiver operating curve
SD	standard deviation
TEN-T	Tris (hydroxymethyl) methylamine EDTA and NaCl with 0.05% (v/v) Tween 20
TEN-TC	TEN with 0.05% (v/v) Tween 20 and 0.2% (w/v) casein
TG-ROC	computer program for the calculation of a cut-off for the ELISA using two graph response-operating characteristic curves
WA	Western Australia
w/v	weight in volume (%)
v/v	volume in volume (%)