ACTIVE DISEASE SURVEILLANCE IN KANGAROOS UTILISING THE COMMERCIAL HARVESTING INDUSTRY

Presented by

Abbey Potter, BSc. BVMS (Hons)

Submitted in total fulfilment of the requirements of the degree of

Doctor of Philosophy

School of Veterinary and Biomedical Sciences

Department of Public Health and Epidemiology

Division of Health Sciences

Murdoch University

Western Australia

July, 2011

Declaration

This thesis constitutes work carried out by the candidate unless otherwise stated and does not incorporate without acknowledgement any material previously submitted for a degree at any tertiary institution. The thesis is less than 100,000 words in length, exclusive of tables, figures, bibliography and appendices and complies with the stipulations set out for the degree if Doctor of Philosophy by Murdoch University.

Abbey Potter, BSc. BVMS (Hons)

School of Veterinary and Biomedical Sciences

Murdoch University

Murdoch, Western Australia 6150

Australia

Table of Contents

List of Tables

List of Figures

Acknowledgements

The work presented here was conducted using funding from the Australian Biosecurity cooperative research centre (ABCRC) and Murdoch University. Experiments crucial to this study were performed in the Arbovirus Surveillance and Research Laboratory at the University of Western Australia, the State Agricultural Biotechnology Centre (SABC) at Murdoch University and the Animal Health Laboratory at the WA Department of Agriculture and Food.

I would like to thank each of my supervisors A/Prof. Stan Fenwick, Dr. Simon Reid, Dr. Cheryl Johansen and Dr. Michael Lindsay for their undying support, enthusiasm and encouragement over the course of this research. Your patience was a virtue! To the girls from the Arbovirus Surveillance and Research Laboratory, your assistance in the laboratory was invaluable as has been the friendship we have developed.

I would like to thank the kangaroo shooters (and their partners), processor employees and industry representatives at DEC who each donated their time, assistance and knowledge to this project. The hospitality and midnight muffins supplied by Professional Shooter A and his wife, were a welcome treat after a long night of sample collecting!

Finally, I would like to make a special mention of Ryan and my family for their undying love and support over the past five years. To my two incredible children, Riley and Layla, who endured this experience with me - it was a challenge little ones, but we did it!

Aims

1. To develop a framework for active disease surveillance in kangaroos using the commercial harvesting industry.

2. To determine the prevalence of naturally acquired *Salmonella* infection in wild kangaroos in Western Australia.

3. To further define the role of the kangaroo as reservoir host of *Coxiella burnetii* in Western Australia.

4. To further define the role of the western grey kangaroo as reservoir host of Ross River virus and determine whether ongoing surveillance will improve the capacity to predict periods of increased viral activity.

Thesis Abstract

The aim of this study was to develop a framework for disease surveillance in one of the Australia's most abundant macropods using the kangaroo harvesting industry. The impetus for this work arose because wildlife species are considered to play a significant role in the introduction, maintenance and spread of a majority of the world's emerging infectious diseases yet active disease surveillance is rarely undertaken in these free-ranging populations. The framework developed was trialled by collecting samples and testing them for a number of significant emerging infectious diseases, including *Salmonella, Coxiella burnetii* and Ross River virus (RRV).

Kangaroos have long been suspected of carrying high levels of *Salmonella,* yet no definitive study has been undertaken to determine the true prevalence of infection in their natural habitat. Faecal samples were collected from 645 western grey kangaroos (*Macropus fuliginosus*) from ten different geographical locations throughout Western Australia over a period of 18 months and cultured for *Salmonella* spp. The estimated prevalence in the animals surveyed was approximately 3.6%. Faecal shedding was greatest following increased periods of rainfall in the April to June quarter. The relatively low prevalence of faecal shedding suggests that kangaroos in their natural habitat support the organism but are unlikely to pose any greater risk of zoonotic infection than other domestic livestock species. Whilst kangaroos have not yet been associated with food-borne outbreaks of disease, serotypes known to cause salmonellosis were isolated in this study, such as *Salmonella enterica* serovar Muenchen, Kiambu and Saintpaul.

Few studies have investigated the role of macropods in the maintenance and transmission of *C. burnetii*. Paired faecal and serum samples were collected from approximately 1000 western grey kangaroos from across twelve locations throughout Western Australia. An indirect ELISA was used to detect *C. burnetii* antibodies in serum, whilst quantitative PCR was used to detect *C. burnetii* DNA in faecal material. The estimated seroprevalence across all sample collection sites was 24.1%, whilst *C. burnetii* DNA was detected in the faeces of 4.1% of animals surveyed. Seroprevalence was significantly higher following increased periods of rainfall in the 60 days prior to sample collection ($p<0.05$), with seroprevalence lowest in the October to December quarter. These results suggest that kangaroos are likely reservoirs of the organism in Western Australia, posing a zoonotic threat to industry workers and animal handlers.

Ross River virus is Australia's most common mosquito-borne disease and the western grey kangaroo is suspected of being a significant vertebrate host in the southwest of Western Australia. A total of 2605 serum samples, collected from across fourteen locations throughout the state, were tested for RRV neutralising antibodies. The seroprevalence varied significantly between geographical regions but was estimated to be 44.0% across all sample collection locations. Despite difficulties associated with age-based selection bias introduced through the kangaroo harvesting industry, surveillance within western grey kangaroo populations appears to provide a means of assessing the background risk of RRV for any given location and may assist in improving the capacity to predict future RRV activity.

Abbreviations

<	less than
>	greater than
≤	less than or equal to
≥	greater than or equal to
±	plus or minus
%	percent
°C	degrees Celcius
µl	microlitre
AB-CRC	Australian Biosecurity Cooperative Research Centre
ABS	Australia Bureau of Statistics
ADE	antibody dependent enhancement
Apr	April
ASRL	Arbovirus Surveillance and Research Laboratory
AWHN	Australian Wildlife Health Network
BOM	Bureau of Meteorology
CALM	Conservation and Land Management
CFT	complement-fixation test
CI	confidence interval
cm^2	centimetres squared
Dec	December
DEC	Department of Environment and Conservation
dd	double distilled
DNA	deoxyribonucleic acid
EDTA	ethylenediamine-tetra acetic acid, tri-potassium salt
EID	emerging infectious disease
ELISA	enzyme-linked immunosorbent assay
et al.	and others
EVS	encephalitis vector survey
FBS	foetal bovine serum
FMD	Foot and mouth disease
g	grams
g	unit of gravitation field

G	gauge
GIS	geographical information system
H_2O	water
HI	haemagglutination-inhibition
IgA	immunoglobulin A
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
Jan	January
Jun	June
Jul	July
kg	kilogram
KMAC	Kangaroo Management Advisory Committee
L	litre
LCV	large cell variant
m	metre
M	molar concentration
Mar	March
MBDC	Mosquito-Borne Disease Control Branch (Department of Health)
min	minute
MIR	minimum infection rate
ml	millilitre
mm	millimetre
n	number of animals
NC	not calculated
ND	not detected
NDVI	normalised difference vegetation index
NEPSS	National Enteric Pathogens Surveillance Scheme
NSW	New South Wales
NT	neutralisation test
NTC	no template controls
Oct	October
OD	optical density
OR	odds ratio

PP	percent positive
QLD	Queensland
qPCR	quantitative polymerase chain reaction
RainCat30	accumulated rainfall in the previous 30 days
RainCat60	accumulated rainfall in the previous 60 days
RRV	Ross River virus
SA	South Australia
Sept	September
SCV	small cell variant
SDC	small dense cell
$TCID_{50}$	50% tissue culture infectious dose
TEN-T	TE and NaCl with 0.05% (v/v) Tween 20
VIC	Victoria
v/v	volume in volume
WA	Western Australia
WGK	wester grey kangaroo (*Macropus fuliginosus*)
w/v	weight in volume