TRAPPING OF FREE-LIVING, UNFED ADULT AND NYMPHAL *Amblyomma hebraeum* IN HEARTWATER ENDEMIC AREAS OF SOUTH AFRICA AND THE PREVALENCE OF *Cowdria ruminantium* IN A SAMPLE OF ADULT TICKS

by

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Submitted in partial fulfilment of the requirements for the degree of

MASTER IN VETERINARY MEDICINE (PARASITOLOGY)

IN THE FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

PRETORIA

2000

DEDICATION

To my parents who believed

in the

importance of education,

and to

Mark and Danielle

for their support.

DECLARATION

Apart from the assistance received,

which has been reported in the Acknowledgements,

and in appropriate places in the text,

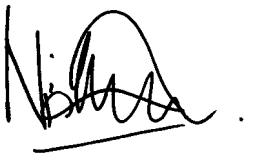
this Dissertation represents the original work of

the author.

The investigations in this Dissertation

have not been presented

for any other degree at any other University.



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ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and thanks to the following people:

Prof. I G Horak - for guidance, support and encouragement, especially during the difficult years of the project

Dr S M Mahan, UF/USAID/SADC Heartwater Project, for having the confidence to guide me away from non-productive research, and for his whole-hearted support during this project

Prof. E H Venter - for assistance with the administration of the project, particularly the molecular biological aspects

Prof. C G Stewart - for kindling my initial interest in heartwater, and for the many discussions on all aspects of research

Mr B Simbi, UF/USAID/SADC Heartwater Project, for many hours of instruction in PCR laboratory techniques, and for demonstrating how PCR should be done

Dr T F Peter - for his assistance in PCR techniques, and discussions on tick epidemiology

Prof. A S Greeff and **Dr C C Clay** - for assisting me with my first steps into understanding molecular biology

Prof. P J Kelly and **Dr L Mathewman**, Department of Clinical Studies, Faculty of Veterinary Science, University of Zimbabwe, Harare, for introducing me to the UF/USAID/SADC Heartwater Project in Harare

Dr G A Tice and **Dr C E Yunker** - for the initial help and discussions on the use of the AAAP/CO_2 trap in the field

Dr S W Vogel - for discussions on the project, and assistance with obtaining publications

Dr & Mrs P van der Riet - for accommodation and transport in East London, and the **Staff Members** at the Veterinary State Office, East London, for their assistance

Dr C de Bruin, **Dr T Strydom** and other **Staff Members** of the SABS - for access to the farm , and assisting me with the collection of the ticks

Prof. E F Donkin - for discussions and proof reading

Prof. J A W Coetzer - for supporting the project

Dr L E O Braack, **Dr R G Bengis** and their **Staff** - for accommodation and transport, whilst staying at Skukuza, Kruger National Park

Mr A M Spickett - for discussions on various aspects of the Kruger National Park

Dr J E Crafford - for many hours of assistance with computer graphics and colour printing

Prof. K P Pettey - for all his assistance with the scanner and computer graphics

Dr L van der Merwe - for once again involving herself in tick research projects

Prof. H S Schoeman - for assisting me with the statistical approach to the project

Prof. B Gummow - for advice on epidemiology and statistics

Dr B H du Plessis, Mpumalanga Veterinary Department - for data on the Songimvelo Game Reserve

Mr H M Ebrahim and **Mr R L Carr** - for years of ongoing help with photographic and other audio-visual assistance

Mr & Mrs O Coltman, Sable Ranch, Brits - for encouragement and accommodation during the project

Mr & Mrs N Travlos, Harare, Zimbabwe - for accommodation and their hospitality during my stay in Harare

Ms C du Toit - for supplying me with dry ice

Mrs A van den Berg - for giving superb secretarial support

The **UF/USAID/SADC Heartwater Project** for financial assistance, and the use of the laboratory in Harare where all the PCR analysis was conducted

The **FRD (UDP) Programme** - for financing a major part of the project

The **University of Pretoria (Department of Veterinary Tropical Diseases)**, and **MEDUNSA (Department of Infectious Diseases & Public Health)**, are thanked for their financial contributions.

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Trapping of free-living, unfed adult and nymphal *Amblyomma hebraeum* in heartwater endemic regions of South Africa, and the prevalence of *Cowdria ruminantium* in a sample of adult ticks

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PROMOTERS: PROFESSOR E. H. VENTER

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ABSTRACT

The main objective of this study was to use the Attraction-aggregation-attachment-pheromone/carbon dioxide (AAAP/CO_2) trap on a sustainable basis at six different field sites in South Africa. This trap was developed in Zimbabwe, but had not been used successfully in the field for the collection of free-living adult and nymphal *A. hebraeum*.

A two-year collection survey was carried out at one of the sites, the Rietgat communal grazing area (CGA) where 1 196 adult and 292 nymphal *A. hebraeum* were trapped with the AAAP/CO_2. Only free-living, unfed adult and nymphal *A. hebraeum* were collected, as these ticks were considered to be epidemiologically more credible than ticks collected off hosts. A distinct seasonal appearance of adult ticks was noted in both 1996 and 1997, and this could explain the difficulty experienced in collecting these ticks in the field in the past.

Peak numbers of adult ticks were collected from late spring (September/October) to midsummer (November - January). This was followed by a sharp decline to very low counts for the remainder of the year (February - August).

Field work was also conducted at five other sites in South Africa. At three of these sites, the AAAP/CO_2 trap was used successfully, these included a farm near East London (n = 187 adults, 17 nymphs) Kruger National Park (KNP) (n = 447 adults) and the Songimvelo Game Reserve (SGR) (n = 48 adults). At the two other sites, namely the MEDUNSA campus (n = 31 adults) and at a farm near Warmbaths (n = 25 adults), the AAAP/CO_2 trap was not really successful. A total of 1 934 adult and 309 nymphal *A. hebraeum* were collected with the AAAP/CO_2 trap.

A sample (n = 570) of the adult ticks collected from the Rietgat CGA (n = 434), the KNP (n = 88) and the SGR (n = 48) was tested for *C. ruminantium* with a specific PCR assay developed at the UF/US AID/SADC Heartwater Research Project in Harare, Zimbabwe. Nearly nine per cent (8.9%) of the ticks from the Rietgat CGA, 5.7% from the KNP and 25% from the SGR were positive for *C. ruminantium*. The overall infection rate of 9.8% for the total sample (n = 570) is similar to others recorded in southern Africa.

This was the first time that a large, statistically-relevant sample of free-living, unfed adult *A. hebraeum* collected with a AAAP/CO_2 trap, from a variety of different ecological areas has been processed with a *C. ruminantium*-specific PCR. The epidemiological data from this project should be more credible than those from many of the previous surveys, where feeding ticks were collected off hosts, and indirect methods used to determine *C. ruminantium* prevalence.

Vang van vrylewende, ongevoede volwassenes en nimfe van *Amblyomma hebraeum* in inheemse hartwatergebiede van Suid Afrika, en die teenwoordigheid van *Cowdria ruminantium* in a monster van volwasse bosluise

N R BRYSON

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DR S. M. MAHAN

SAMEVATTING

Die hoof doel van hierdie studie was om die "Attraction-aggregation-attachment-pheromone ($AAAP/CO_2$)" lokval op 'n volgehoue grondslag op ses verskillende terreine in die veld te gebruik. Hierdie lokval wat in Zimbabwe ontwikkel is, is nog nie met sukses vir die versameling van vrylewende, ongevoede *A. hebraeum* in die veld gebruik nie.

Tydens 'n twee-jarige ondersoek by een van hierdie terreine, die Rietgat gemeenskaps-weiding, is 1 196 volwassenes en 292 nimfe van *A. hebraeum* met die $AAAP/CO_2$ lokval versamel. Slegs vrylewende, ongevoede *A. hebraeum* is versamel, aangesien hierdie bosluise epidemiologies meer geloofwaardig beskou word as bosluise wat vanaf gashere versamel is. 'n Duidelike seisoenale patroon in volwasse bosluisgetalle het gedurende 1996 sowel as 1997 voorgekom, en dit kan moontlik verklaar waarom daar in die verlede probleme ondervind is met die versameling van hierdie bosluise. Die grootste getalle bosluise is in die laat lente (September/Oktober) tot middel somer (November - Januarie) versamel, gevolg deur 'n skerp daling in getalle gedurende die res van die jaar (Februarie - Augustus).

Die versameling van bosluise is ook in vyf ander gebiede in Suid Afrika uitgevoer. Die AAAP/CO_2 lokval is in die volgende drie gebiede suksesvol gebruik: SABS plaas, Oos London (n = 187 volwassenes, 17 nimfe), Nasionale Kruger Wildtuin (n = 447 volwassenes) en Songimvelo Wildreservaat (n = 48 volwassenes). By die MEDUNSA kampus (n = 31 volwassenes) en die Warmbad plaas (n = 25 volwassenes) was die AAAP/CO_2 lokval minder suksesvol. 'n Totaal van 1 934 volwassenes en 309 nimfe is met die AAAP/CO_2 lokval versamel.

'n Monster (n = 570) van die volwasse bosluise wat by die Rietgat (CGA) gemeenskaps-weiding (n = 434), die Nasionale Kruger Wildtuin (n = 88) en Songimvelo Wildreservaat (n = 48) versamel is, is vir die teenwoordigheid van *C. ruminantium* met behulp van 'n PCR metode getoets. Hierdie toets is ontwikkel deur die UF/USAID/SADC Hartwater Navorsingsprojek in Harare, Zimbabwe. Byna nege persent (8.9%) van die bosluise wat by die Rietgat gemeenskapsweiding, 5.7% in die Nasionale Kruger Wildtuin en 25% in die Songimvelo Wildreservaat versamel is, was positief vir *C. ruminantium*. Die algehele persentasie besmetting van die totale aantal bosluise (n = 570) was 9.8%, en is soortgelyk aan die in ander gebiede in suidelike Afrika.

Dit is die eerste grootskaalse, statisties-aanvaarbare monster van vrylewende, ongevoede volwasse *A. hebraeum* wat met die gebruik van die AAAP/CO_2 lokval versamel is in 'n verskeidenheid ekologiese areas, en met 'n *C. ruminantium*-spesifieke PCR getoets is. Die epidemiologiese data wat verkry is met hierdie projek, behoort meer geloofwaardig te wees as enige vorige opnames waar gevoede bosluise vanaf gashere versamel is, en indirekte metodes gebruik is om die teenwoordigheid van *C. ruminantium* te bepaal.

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ABBREVIATIONS

AAAP/CO_2	Attraction-Aggregation-Attachment-Pheromone/Carbon dioxide
ASF	African swine fever
ASFV	African swine fever virus
α^{32}P (dCTP)	Alpha phosphorus (32) deoxycytidine triphosphate
BAE	Bovine aortic endothelial
bp	Base pair(s)
c-ELISA	competitive-Enzyme-linked Immunosorbent Assay
CGA	Communal grazing area(s)
CO_2	Carbon dioxide
°C	Degrees Celsius
DNA	Deoxyribonucleic Acid
dATP	2'-deoxyadenosine-5'triphosphate
dCTP	2'-deoxycytidine-5'triphosphate
dGTP	2'-deoxyguanosine-5'triphosphate
dNTP	2'-deoxynucleoside-5'triphosphate
dTTP	2'-deoxythymidine-5'triphosphate
ECF	East Coast fever
ELISA	Enzyme-linked Immunosorbent Assay
FAT	Fluorescent antibody test
fg	Femtogram(s)
Fig.	Figure

g	Gram
i-ELISA	indirect-Enzyme-linked Immunosorbent Assay
IFAT	Indirect fluorescent antibody test
I/P	Intraperitoneally
I/V	Intravenously
Kb	Kilobase
Kbp	Kilobase pair(s)
KCl	Potassium chloride
kDa	Kilodalton
kg	Kilogram(s)
KNP	Kruger National Park
MAP	Major Antigenic Protein
MEDUNSA	Medical University of Southern Africa
mg	Milligram(s)
MIA	Mouse Infectivity Assay
$MgCl_2$	Magnesium chloride
mℓ	Millilitre(s)
mm	millimetres
mM	Millimolar
N	Normal
NaoH	Sodium hydroxide
ng	Nanogram(s)

ORF	Open Reading Frame
^{32}P	Phosphorus 32
P	Prevalence
PBS	Phosphate buffered saline
PCIA	Phenol:chloroform-isoamyl-alcohol
PCR	Polymerase Chain Reaction(s)
pCR9	Kenyan heartwater probe
pCS20	Zimbabwean heartwater probe
pg	Picogram(s)
RH	Relative humidity
RNA	Ribonucleic Acid
r-RNA	Ribosomal RNA
RVF	Rift Valley fever
SABS	South African Bureau of Standards
s/c	Subcutaneously
SDS	Sodium dodecyl sulphate
SGR	Songimvelo Game Reserve
TAR	Tick attack rate
TBD	Tick-borne diseases
TID	Tick infectivity decay
Tris HCl	Tris (hydroxymethyl) aminoethane

UF/USAID/SADC	University of Florida/United States Agency for International Development/Southern African Development Community
μg	Microgram(s)
$\mu\ell$	Microliter(s)
μM	Micromolar
UV	Ultraviolet
w/v	weight/volume.