EPIZOOTOLOGICAL STUDIES AND DIAGNOSTIC APPROACHES TOWARDS CATTLE BRUCELLOSIS IN THE SMALLHOLDER DAIRY SECTOR OF ZIMBABWE

A thesis presented by

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In partial fulfilment of the requirements for the degree of Doctor of Philosophy (D.Phil.)

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DECLARATION

1. **BY THE CANDIDATE**

The work presented in this thesis is to the best of my knowledge and belief, original, and does not contain any material published elsewhere, except where reference is made. This material has not been submitted, either in part or whole, for degree or diploma at this or any other university.

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This thesis has been submitted for examination with our approval as University supervisors.

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ABSTRACT

A cross-sectional study was conducted to study the epizootology and diagnostic approaches of cattle brucellosis in six smallholder cattle farming areas of Zimbabwe. Specifically, the seroprevalence and risk factors for brucellosis were investigated. Serological diagnostic tests were evaluated and bacteriological investigations of herds and characterisation of Brucella spp. were carried out.

The overall mean individual animal and herd-level seroprevalences were 5.6% (95% CI: 4.4%, 6.8%) (81/1440) and 25.0% (95% CI: 18.1%, 31.9%), (52/203) respectively. The seroprevalence differed significantly (P<0.05) among the study areas. Young animals (2-4 years) were found to be 5 times (OR=5.0, 95% CI: 1.4, 16.7) more likely to be positive compared to old animals (>7 years). Animals in the age group 5.5-7 years were found to be approximately 5 times (OR=4.9, 95% CI: 2.0, 11.6) more likely to have aborted compared to those of the age group 2-4 years, but the risk subsequently decreased with increasing age. Keeping mixed cattle breeds was associated with increased risk of brucellosis (OR= 8.5; 95% CI: 2.7, 26.5). Seropositivity (OR = 3.0, 95% CI: 1.4, 6.6) and mixed breed herds (OR = 2.3, 95% CI: 1.1, 4.9) were respectively found to be associated with increased risk of abortions. The kappa statistic test indicated good agreement among the c-ELISA, RBT and the FPA. The FPA had a higher specificity compared to RBT. The biochemical profiles of the B. abortus biovar 1 (11 isolates) and biovar 2 (2 isolates) typed in this study were typical of those of the genus. In conclusion, brucellosis was present in all study areas. The age of cattle and the mixing cattle breeds are important risk factors for brucellosis. The FPA could be used as a confirmatory test for bovine especially in the field. It is likely that B. abortus biovar 1 is the predominant cause of brucellosis in smallholder cattle. Further tests are required to study molecular biology and the epizootology of B. abortus.
This project was carried out as a nested programme, within the Department of Paraclinical Veterinary Studies at the University of Zimbabwe and the Department of Infection Biology at the Norwegian School of Veterinary Science in Oslo. The project was sponsored by the Norwegian Council for Higher Education and Development (NUFU) who also sponsored similar projects on zoonotic infections and environmental toxicology in Tanzania, Uganda, Zambia, Mozambique and South Africa. The main aim of these projects was to foster linkages and enhance collaborative research among the African regional universities and institutions in Norway, namely the Norwegian School of Veterinary Science and the National Veterinary Institute. In these projects, the Universities of Zambia and Zimbabwe were tasked to carry out studies on bovine brucellosis as an important zoonosis, while Makerere University was researching on bovine tuberculosis. The University of Pretoria, Eduardo Mondlane University and Sokoine University of Agriculture were working on environmental toxicology.

I am indebted to my principal supervisor, the late Professor Krishna Mohan, whose vast experience and knowledge on bovine brucellosis was sorely missed. I wish to express my sincere gratitude to my current supervisors; Professors Eystein Skjerve and Evison Bhebhe for their critique, mentorship and encouragement that culminated in the compilation of this thesis. The project would not have been a success without the professional contribution of Dr Arve Lund of the National Veterinary Institute, Oslo, Norway.

I specifically thank Dr Berit Djønne of Veterinary Institute, Oslo for allowing me access to the laboratory facilities for characterisation of Brucella isolates. The training and guidance accorded to me by all members of staff at the institute is highly appreciated.
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It was my pleasure to have worked with fellow postgraduate students, John Bwalya Muma (Zambia), James Oloya (Uganda) in the “Zoonoses” component of the NUFU project. John deserves special mention for his invaluable critique and comments on my thesis manuscript. I thank him for the moral support he gave me whilst I was working on the thesis in Lusaka.

Last, but not least, I wish to express my appreciation to my wife Florence, daughter Sandra, sons Kumbirai and Gainmore, for their patience and understanding during the long hours they spent without me.
DEDICATION

This work is dedicated to my first principal supervisor, the late Professor Krishna Mohan. Having taught me the sequential primary and secondary tests used to identify the genus *Brucella*, he coined the phrase “pure honey drop-like”, as a perfect description of the colonies of *Brucella abortus* on Mueller-Hinton agar. After we had gone through all the identification procedures, he said to me; “if you master your subject, you will never be caught wanting”.
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LIST OF ABBREVIATIONS

AGID = Agar gel immunodiffusion test
AMOS-PCR = \textit{Brucella abortus, Brucella melitensis, Brucella ovis, Brucella suis},
\hspace{1em} polymerase chain reaction
ARDA = Agricultural Rural Development Authority
AUC = Area under a curve
c-ELISA = Competitive enzyme-linked immunosorbent assay
CFT = Complement fixation test
CI = Confidence interval
DNA = Deoxyribonucleic acid
FPA = Fluorescence polarization assay
HIV/AIDS = Human immunodeficiency virus/Acquired immunodeficiency syndrome
i-ELISA = Indirect enzyme-linked immunosorbent assay
IR = Incidence rate ratio
IU = International units
LPS = Lipopolysaccharides
METAT = 2-Mecarptoethanol tube agglutination test
mP = Millipolarisation units
MRT = Milk ring test
OPS = O-polysaccharides
OR = Odds ratio
PCR = Polymerase chain reaction
PI = Percent inhibition
RBT = Rose Bengal plate test
RLPS = Rough Lipopolysaccharides
RNA = Ribonucleic acid
ROC = Receiver operator characteristic curves
SAT = Serum agglutination test
SE = Standard error
TSB = Tryptone Soya broth
VNTR = Variable number of tandem repeat analysis