Epidemiology and molecular confirmation of *Brucella* spp. in cattle in Namibia

by

Oscar Madzingira

A thesis

submitted in fulfilment of the requirements for the degree

Doctor of Philosophy

in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science,

University of Pretoria

Supervisor:

Prof Henriette van Heerden

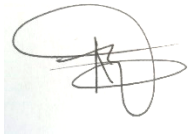
Co-supervisor:

Prof Folorunso O Fasina

March 2021

Declaration

I declare that the thesis, which I submit for the degree **Doctor of Philosophy (Ph.D.)** at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.



_____

Oscar Madzingira
March 2021

Acknowledgements

To my supervisor, Prof. Henriette van Heerden, thank you for the constant encouragement, material and academic support which made the project a success. The two-week fully funded laboratory training at Onderstepoort prior to the commencement of this project was valuable. It was a pleasure working under your supervision. To my co-supervisor, Prof. Folorunso O. Fasina, thank you for your continuous guidance and encouragement throughout. You were there when needed.

I am grateful to the University of Pretoria for financial support through the UP Postgraduate Bursary, which made it easier to manage research expenses.

My appreciation goes to the Ministry of Health and Social Services and the Ministry of Agriculture, Water and Forestry, Namibia, for permitting this study and for providing access to data that was used as part of the project. The Chief Medical Officers of Hardap, Khomas and Zambezi regions assisted with arrangements for the distribution of questionnaires among medical doctors and nurses. Many thanks to the Chief Veterinary Officer (Dr. A. Shilongo) for permitting the study at the government regulated abattoir and the Central Veterinary Laboratory. Dr. S. Khaiseb and his team at the Central Veterinary Laboratory (Windhoek) provided access to the laboratory, materials, and technical support during the performance of serological and molecular assays. Many thanks to the respondents who took time off their busy schedules to participate in the questionnaire study.

I am grateful to the study abattoir management for permitting part of the study (sample collection) to be carried out on their premises and the abattoir state veterinarian, Dr. F. Kandongo and his team for logistical support rendered during sampling and questionnaire interviews.

Kind thanks to Dr. Francis B. Kolo for support rendered during the isolation and characterisation of *Brucella* species and for mentorship in the bacteriology and molecular laboratories during my two-week visit. Dr. C. Byaruhanga provided

valuable and much needed support during the analyses of data from the questionnaire study. University administrative staff, in particular, Ms. Rina Serfontein, Marieka Schoeman, Leanne van Zyl, Leonie Johnson, Rachel Coetzee and many others, made it very easy to navigate through the administrative processes of my study.

Many thanks to my family (Bea, Runya, Titi, Ruru) for your patience while I focussed on this work.

To God be the glory, honour, and praise for the grace to complete this project.

Dedication

To my beloved family, daughters Ruvimbo, Runyararo, son Tinaye and wife Beatitude.

Table of contents

List of Figures

List of Tables

List of Abbreviations

AIDS	Acquired Immunodeficiency syndrome
AMOS-PCR	PCR for the identification of *Brucella abortus*, *B. melitensis, B. ovis* or *B. suis*
bp	Base pairs
BSL 2+	Biosafety Level 2+
BST	Brucellin skin test
c-ELISA	Competitive enzyme-linked immunosorbent assay
CFT	Complement Fixation Test
CI	Confidence interval
COVID	Corona Virus Disease
DNA	Deoxyribonucleic acid
EC	European Commission
EDTA	Ethylenediaminetetraacetic acid
EU	European Union
FAO	Food and Agriculture Organisation
FITC	Fluorescein isothiocyanate
FPM	Fluorescence polarisation analyser

FM	Farell’s medium
FPA	Fluorescence polarisation assay
GIS	Geographic information system
HCPC	Hierarchical Clustering on Principal Components
HIV	Human immunodeficiency virus
i-ELISA	Indirect enzyme-linked immunosorbent assay
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ITS	Intergenic transcribed spacers
KAP	Knowledge, attitudes, and practices
KDa	Kilodalton
Kg	Kilograms
Km	Kilometre
LPS	Lipopolysaccharides
M cells	Microfold cells

MAWF	Ministry of Agriculture, Water and Forestry
MCA	Multiple correspondence analysis
Mg	Milligrams
Mm	Millimetres
mTM	Modified Thayer-Martin
MRT	Milk Ring Test
MLVA	Multiple-locus VNTR analysis
NCA	Northern Communal Areas
NIP	Namibia Institute of Pathology
^{o}C	degrees Celsius
OIE	World Organisation for Animal Health
OPS	O-Polysaccharide
PCR	Polymerase chain reaction
pH	potential of hydrogen
RBT	Rose Bengal test
RFLP	Restriction fragment length polymorphism
rpm	revolutions per minute

RR	Relative risk
rRNA	Ribosomal ribonucleic acid
SAT	Serum Agglutination Test
SDA	Serum dextrose agar
SSA	sub-Saharan Africa
spp.	Species
TSA	Tryptose soy agar
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
VCF	Veterinary cordon fence
VNTR	Variable number tandem-repeats
VP	Voges-Proskauer
WHO	World Health Organisation
WGS-SNP	Whole Genome Sequencing-Single Nucleotide Polymorphism
µm	Micrometre

Epidemiology and molecular confirmation of *Brucella* spp. in cattle in Namibia

Candidate: Oscar Madzingira

Supervisor: Prof Henriette van Heerden

Co-supervisor: Prof Folorunso O Fasina

Department: Veterinary Tropical Diseases

Degree: PhD

Thesis Summary

This study assessed knowledge, attitudes, and practices regarding brucellosis among cattle farmers (n=264), meat handlers (n=143) and medical professionals (n=124); estimated seroprevalence of *Brucella* infection in cattle (n=49718) (2004-2018) and humans (n=971) (2012-2017) retrospectively, and prospectively (n=304) at a major abattoir in Namibia. Molecular characterisation of *Brucella* species was performed on DNA extracted from spleen and lymph nodes from seropositive cattle. Overall awareness of brucellosis was 43.50% (231/531), with highest awareness among medical professionals (73.40%, 91/124) and the lowest in meat handlers (14.00%, 20/143). Medical professionals (98.40%, 122/124) did not consider brucellosis in the differential diagnosis of persistent fever in humans. Seroprevalence of human brucellosis was 11.64% (113/971, 95% CI: 9.77-13.81), with positive cases clustered in the 30-40-year age group and in females (64.00%) ($z=-5.24$, $p<0.01$). Individual cattle and herd prevalence of brucellosis was 0.49% (244/49718, 95% CI: 0.43%-0.56%) and 9.26% (78/842, 95% CI: 7.49%-11.41%) respectively, with more seropositive communal herds (33.09%) and cattle (10.27%) than commercial herds (4.67%) and cattle (0.24%) ($p<0.05$). Seroprevalence of brucellosis in the abattoir was 2.30% (7/304; 95% CI: 1.10-4.70%) based on RBT, and 1.64% (5/304; 95% CI: 0.70-3.8%) after confirmation with CFT, while herd prevalence was 9.62% (5/52). *Brucella* DNA was detected in lymph nodes (6/7, 85.71%) and spleens (6/7, 85.71%)

from seropositive cattle using ITS-PCR. From cultures, *Brucella abortus* isolates were confirmed from lymph nodes (4/7, 57.14%) and spleen (6/7, 85.71%) by AMOS-PCR. Targeted public health education, better enforcement of current control measures and the use of protective gear are recommended to prevent human and animal infection.

Chapter 1: General Introduction

1.1. Introduction

Livestock farming is the backbone of the agricultural sector in Namibia. With an estimated population of 2 800 000, cattle production is the major livestock farming activity that contributes approximately 62% to total livestock output in Namibia (Agriculture Statistics Bulletin, 2017). Approximately 70% of cattle are raised on communal grazing land, where different herds intermingle and share grazing, while 30% are reared on private commercial farms (Agriculture Statistics Bulletin, 2017). Most cattle (66.6%) are marketed on hoof to neighbouring countries, while the remainder are slaughtered at export (28.1%) and local abattoirs (5.3%) (Agriculture Statistics Bulletin, 2017). Statistics show that 84 000 cattle were slaughtered at export abattoirs and 24 000 cattle at local abattoirs and butcheries in 2017 (Meat Board of Namibia, 2018). For sustained livestock and beef exports and foreign currency earnings, a sound sanitary environment in which the major infectious and zoonotic diseases such as brucellosis are controlled in livestock is mandatory.

Brucellosis is a highly infectious zoonotic disease of great public health and economic importance that affects both humans and domestic animals in many regions of the world (Alton, 1991). The disease is caused by bacteria belonging to the genus *Brucella*, with *B. abortus, B. melitensis* and *B. suis* as primary pathogens of cattle, sheep or goats, and pigs respectively (OIE, 2018). *Brucella* infection has negative impacts on animal health and production (Godfroid et al., 2005) through abortions, retained placentas, reduced milk production, culling of infected animals and restrictions imposed on local or international trade, orchitis, epididymitis, infertility and arthritis (FAO, 2006; OIE, 2018). The disease persists in pastoral communities due to limited knowledge of the epidemiology of the disease and insufficient resources and capacity for local diagnosis (McGiven, 2013; Racloz et al., 2013). Veterinary authorities often place more emphasis on the control of the highly infectious diseases of economic importance such as Foot-and-Mouth disease (Hadush & Pal, 2013). The World Organisation for Animal Health (OIE) recognizes

the importance of this disease in relation to international trade and therefore member states are required to report incidence of the disease on a regular basis.

Human brucellosis is a disease of significant social, economic, and medical importance (FAO, 2006) and an occupation-associated disease, with the abattoir workers, farm workers, butchers, laboratory personnel, veterinarians, and veterinary paraprofessionals at a greater risk of infection (EC, 2001; WHO, 2005). It is a disease that is often overlooked (Hotez and Gurwith, 2011), and under-reported (Crump et al., 2013) by public health authorities in developing countries despite its contribution to disease burden in livestock-dependent areas of Africa (Fèvre et al., 2017). In sub-Saharan Africa (SSA), the presence of malaria as a cause of acute febrile illness is reported to confuse the diagnosis of brucellosis (Crump et al., 2013; Stoler and Awandare, 2016). Therefore, brucellosis is a neglected (Hotez and Gurwith, 2011) and re-emerging disease (Pappas, 2010). Globally, about half a million cases of human brucellosis are reported annually (Pappas, 2010), and this is thought to be an underestimate (WHO, 2006). Global incidence of the disease has been reported to vary widely from <0.09 to >10 per 100 000 population (Taleski et al., 2002; Pappas et al., 2006; Seleem et al., 2010). In South Africa, annual incidence of <0.1 to 0.3 per 100 000 population has been reported (Küstner, 1985).

Although brucellosis is endemic in Africa, disease data from bacteriological and DNA analyses is limited (Whatmore et al., 2016). Therefore, in parts of Africa where the infection occurs, it is poorly described epidemiologically and genetically (Foster et al., 2018). Most studies of brucellosis in cattle are based on serological surveys using the Rose Bengal test (RBT), Complement Fixation Test (CFT) or the enzyme linked immunosorbent assay (ELISA) singly or in combination (Ducrotoy et al., 2017), which provide limited understanding of the molecular epidemiology of the circulating strains. Although culture and isolation are the universally accepted gold standard method for the detection of *Brucella*, few laboratories have the biosecurity level and capacity to perform the test. As a result, data on the prevalence of *Brucella* infections in populations is often unreliable.

In Namibia, incidence of brucellosis in humans and livestock has not been comprehensively investigated. Species and biovars infecting cattle have not been characterized as serology is mainly used (due to a lack of a biosafety 2+ laboratory), which only allows for the identification of smooth *Brucella* to genus level. Understanding the distribution of biovars is important in ascertaining the source of infections and for managing disease outbreaks and setting up efficient preventive and control programs (Garin-Bastuji et al., 2006; Lucero et al., 2008). Assuming that the species infecting cattle is *B. abortus* may not always be true because in other areas where cattle are reared with sheep and/or goats, *B. melitensis* has been isolated (Godfroid et al., 2013).

Brucellosis was first reported in Namibia in 1953 based on serological tests (Godfroid et al., 2004). Reports from veterinary services and serological studies in cattle, sheep and goat populations (Madzingira and McCrindle, 2014; 2015; 2016; Madzingira and Sezuni, 2017) indicate that brucellosis is an endemic disease in livestock. The most recent outbreak of the disease in Namibia affected both sheep and humans on one farm in the southern part of the country (Magwedere et al., 2011). Brucellosis is a notifiable disease in Namibia whose control is based on the Animal Health Act (Act 1 of 2011) and its associated regulations. Measures for the control of the disease include compulsory vaccination of heifers between 3 and 8 months of age with an approved vaccine (*B. abortus* S19), appropriate identification of infected animals, enforcement of quarantine measures, import restrictions and supervised culling of brucellosis positive cattle. Apart from commercial dairy herds, the slaughter of positive reactor cattle and the testing of cows producing milk for human consumption is not often practiced in other cattle herds, which places the human population at risk of the disease. Moreover, the implementation of brucellosis control measures in communal cattle herds is not strictly enforced and that in small stock is voluntary. Based on personal experience of the researcher, control measures for brucellosis in Namibia have been better implemented and enforced in commercial livestock farming systems especially in dairy cattle farms.

The endemic nature of brucellosis and the fact that cattle slaughtered for human consumption in Namibia are not certified free of brucellosis places meat handlers

and other professions that work with and handle animals and animal products at greater risk of the disease. This is a major concern since the high rate of HIV infection in Namibia may increase susceptibility, disease burden in the population and the severity of clinical manifestations. Moreover, there is insufficient epidemiological data, especially in humans, to support decision making on this re-emerging zoonosis (Marcotty et al., 2009), as has been highlighted by the WHO (2009). In particular, knowledge and awareness of the disease, epidemiology of human brucellosis and the identity of the *Brucella* species infecting cattle in Namibia have not been documented. Therefore, the aim of this study was to use a One Health approach to investigate the epidemiological features of brucellosis in cattle and humans in Namibia including the estimation of prevalence, spatial and temporal distributions, to identify risky practices in humans and characterize *Brucella* species infecting cattle with a view to recommending measures to improve brucellosis control in the country.

1.2. Key Questions

Are farmers, meat handlers and medical professionals aware of brucellosis?

What is the seroprevalence of brucellosis in humans and cattle?

How are brucellosis cases distributed over time and among the regions of the country?

Which *Brucella* species are the cause of infection in cattle in Namibia?

1.3. Aims and objectives

1.3.1. Aim

To describe the epidemiological features of brucellosis in cattle and humans in Namibia with a view to proffer advice to guide the implementation of control measures that can reduce the burden of the disease in humans and cattle.

1.4. Specific objectives

1. To assess knowledge, attitudes and practices related to brucellosis among farmers, meat handlers and medical professionals.

2. To estimate seroprevalence of brucellosis and determine the distribution of positive cases in cattle and humans using secondary data.
3. To estimate the seroprevalence of bovine brucellosis at a major abattoir.
4. To characterise *Brucella* spp. infecting cattle from tissues collected at a major abattoir.

1.5. Thesis overview

Chapter 1: Provides background information and study objectives.

Chapter 2: Gives a review of literature pertaining to bovine and human brucellosis from a global and Namibian perspective.

Chapter 3: Knowledge, attitudes and practices relating to brucellosis were gathered from farmers, meat handlers and medical professionals using questionnaires, with the aim of identifying gaps and positive experiences that can be used to improve brucellosis control in the country.

Chapter 4: Using secondary data, seroprevalence of human brucellosis and active cases was estimated among suspect patients. The association between age, gender, year, region and seropositivity was analysed.

Chapter 5: A retrospective study was carried out to estimate bovine brucellosis seroprevalence and determine the distribution of positive reactors among administrative regions, per year and between cattle production systems in Namibia.

Chapter 6: In this study, seroprevalence of brucellosis was determined at a major cattle abattoir to infer potential risk associated with infection among abattoir workers. Further, the study tested bovine tissues (lymph nodes, spleen, and blood) for *Brucella* spp. DNA using ITS-PCR. Lymph nodes and spleen collected from seropositive cattle were cultured for the isolation and characterization of *Brucella* spp. using AMOS-PCR.

Chapter 7: Discussion, recommendations, and conclusions are detailed based on findings of the entire project.

1.6. References

AGRICULTURAL STATISTICS BULLETIN 2010-2015. 2017. Ministry of Agriculture, Water and Forestry. http://www.mawf.gov.na/documents/37726/764836/2010-2015+AGRICULTURAL+STATISTICS+BULLETIN.pdf/085f71b5-daec-40af-a486-aab5df71a926 Accessed 30 November 2018.

ALTON, G. 1991. *Brucella melitensis*. In Networking in Brucellosis Research. Edited by: Frank JF. Japan, United Nations University Press, 205-216.

CRUMP, JA, MORRISSEY, A.B., NICHOLSON, W.L., MASSUNG, R.F., STODDARD, R.A., GALLOWAY, R.L., OOI, E.E., MARO, V.P., SANGANDA, W., KINABO, G.D., MUIRURI, C. & BARTLETT, J.A. 2013. Etiology of severe non-malaria febrile illness in northern Tanzania: a prospective cohort study. *PLoS Neglected Tropical Diseases*, 7(7), e2324. DOI: 10.1371/journal.pntd.0002324.

DUCROTOY, M., BERTU, W.J., MATOPE, G., CADMUS, S., CONDE-ÁLVAREZ, R., GUSI, A.M., WELBURN, S., BLASCO, J.M. & MORIYÓN, I. 2017. Brucellosis in sub-Saharan Africa: current challenges for management, diagnosis and control. *Acta Tropica*, 165, 179-193. DOI: 10.1016/j.actatropica.2015.10.023.

EC (European Commission). 2001. Brucellosis in sheep and goats *(Brucella melitensis).* https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out59_en.pdf. *Accessed September 23, 2018*.

FAO. 2006. Brucellosis in humans and animals. World Organization in collaboration with the Food and Agriculture Organization of the United Nations and the World Organization for Animal Health; http://www.who.int/csr/resources/publications/Brucellosis.pdf. *Accessed July 28, 2018*.

FÈVRE, E., DE GLANVILLE, W., THOMAS, L., COOK, E., KARIUKI, S. & WAMAE, C. 2017. An integrated study of human and animal infectious disease in the Lake Victoria crescent small-holder crop-livestock production system, Kenya. *BMC Infectious Diseases, 17*, 457. DOI: 10.1186/s12879-017-2559-6.

FOSTER, J.T., WALKER, F.M., RANNALS, B.D., HUSSAIN, M.H., DREES, K.P.,

TILLER, R.V., HOFFMASTER, A.R., AL-RAWAHI, A., KAIM, P. & SAQIB, M. 2018. African lineage *Brucella melitensis* isolates from Omani livestock. *Frontiers in Microbiology*, 8, 2702. DOI: 10.3389/fmicb.2017.02702.

GARIN-BASTUJI, B., VAILLANT, V., ALBERT, D., TOURRAND, B., DANJEAN, M.P., LAGIER, A., RISPAL, P., BENQUET, B., MAURIN, M., DE VALK, H. & MAILLES, A. 2006. Is brucellosis due to the biovar 2 of *Brucells Suisan* emerging zoonosis in France? Two case reports in wild boar and hare hunters. In: Proceedings of the International Society of Chemotherapy Disease Management Meeting. 1st International Meeting on Treatment of Human Brucellosis, 7-10 November, Ioannina, Greece.

GODFROID, J., GARIN-BASTUJI, B., BLASCO, J.M., THOMSON, J. & THOEN, C.O. 2004. *Brucella melitensis* infection. In: COETZER, J.A.W., THOMSON, G.R. & TUSTIN, R.C., editors. Infectious diseases of livestock. Cape Town: Oxford University Press; pp. 1535–1541.

GODFROID, J., CLOECKAERT, A., L. J., KOHLER, S., FRETIN, D., WALRAVENS, K. & LETESSON, J. 2005. From the discovery of the Malta fever‘s agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Veterinary Research, 36*, 313-326. DOI: 10.1051/vetres:2005003.

GODFROID, J., AL DAHOUK, S., PAPPAS, G., ROTH, F., MATOPE, G., MUMA, J., MARCOTTY, T., PFEIFFER, D. & SKJERVE, E.A. 2013. A “One Health” surveillance and control of brucellosis in developing countries: moving away from improvisation. *Comparative Immunology, Microbiology and Infectious Diseases*, 36, 241–248. DOI: 10.1016/j.cimid.2012.09.001.

HADUSH, A. & PAL, M. 2013. Brucellosis—an infectious re-emerging bacterial zoonosis of global importance. *International Journal of Livestock Research, 3*, 28-34. DOI: 10.5455/ijlr.20130305064802.

HOTEZ, P.J. & GURWITH, M. 2011. Europe's neglected infections of poverty, *International Journal of Infectious Diseases*, 15, 611–619. DOI: 10.1016/j.ijid.2011.05.006.

KÜSTNER, H.G.V. 1985. Brucellosis in eastern Orange Free State. *Epidemiological Comments,* 12, 2-23.

LUCERO, N.E., AYALA, S.M. & ESCOBAR, G.I. 2008. *Brucella* isolated in humans

and animals in Latin America from 1968 to 2006, *Epidemiology and Infection*, 136, 496-503. DOI: 10.1017/S0950268807008795.

MADZINGIRA, O. & MCCRINDLE, C. 2014. Prevalence of *Brucella* antibodies in sheep and springbok (Antidorcas marsupialis) reared together in the Karas region, Namibia. *Bulletin of Animal Health and Production in Africa, 62*, 299-306.

MADZINGIRA, O. & MCCRINDLE, C. 2015. Retrospective analysis of the prevalence of *Brucella* antibodies in sheep in the Karas Region of Namibia. *Tropical Animal Health and Production, 47*, 1117. DOI: 10.1007/s11250-015-0838-z.

MADZINGIRA, O. & MCCRINDLE, C. 2016. A questionnaire survey of risk factors of brucellosis on mixed sheep and springbok (Antidorcas marsupialis) farms. *International Science and Technical Journal of Namibia, 8*, 43-49.

MADZINGIRA, O. & SEZUNI, M.P. 2017. Serological prevalence and public health significance of brucellosis on a dairy farm in Namibia from 2011-2014. *BMC Research Notes*, 10, 620. DOI: 10.1186/s13104-017-2933-x.

MAGWEDERE, K., BISHI, A., TJIPURA-ZAIRE, G., EBERLE, G., HEMBERGER, Y., HOFFMAN , L. C. & DZIVA, F. 2011. Brucellae through the food chain: the role of sheep, goats and springbok (*Antidorcus marsupialis*) as sources of human infection in Namibia. *Journal of the South African Veterinary Association, 82*, 205-212. DOI: 10.4102/jsava.v82i4.75.

MARCOTTY, T., MATTHYS, F., GODFROID, J., RIGOUTS, L., AMENI, G., GEY VAN PITTIUS, N., KAZWALA, R., MUMA, J., VAN HELDEN, P., WALRAVENS, K., DE KLERK, L.M., GEOGHEGAN, C., MBOTHA, D., OTTE, M., AMENU, K., ABU SAMRA, N., BOTHA, C., EKRON, M., JENKINS, A., JORI, F., KRIEK, N., MCCRINDLE, C., MICHEL, A., MORAR, D., ROGER, F., THYS, E. & VAN DEN BOSSCHE, P. 2009. Zoonotic tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health issues? The outcomes of a multi-disciplinary workshop. *Annals of Tropical Medicine and Parasitology*, 103, 401-411. DOI: 10.1179/136485909X451771.

MEAT BOARD OF NAMIBIA. 2018. Monthly Statistics. https://www.nammic.com.na/index.php/library/send/51-other/43-monthly-statistics. Accessed September 23, 2018.

MCGIVEN, J.A. 2013. New developments in the immunodiagnosis of brucellosis in livestock and wildlife. *Revue Scientifique et Technique - Office International des Épizooties*, 32, 163-176. DOI: 10.20506/rst.32.1.2205.

OIE. 2018. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 3.1.4. Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis) (infection with B. abortus, B. melitensis* and *B. suis*). http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf. *Accessed February 16, 2019*.

PAPPAS, G.S., PAPADIMITRIOU, P., AKRITIDIS, N., CHRISTOU, I. & TSIANOS, E.V. 2006. The new global map of human brucellosis. *The Lancet Infectious Diseases,* 6, 91-99. DOI: 10.1016/S1473-3099(06)70382-6.

PAPPAS, G. 2010. The changing *Brucella* ecology: novel reservoirs, new threats. *International Journal of Antimicrobial Agents,* 36, S8-S11. DOI: 10.1016/j.ijantimicag.2010.06.013.

RACLOZ, V., SCHELLING, E., CHITNIS, N., ROTH, F. & ZINSSTAG, J. 2013. Persistence of brucellosis in pastoral systems. *Revue Scientifique et Technique - Office International des Épizooties*, 32, 61-70. DOI: 10.20506/rst.32.1.2186.

SELEEM, M.N., BOYLE, S.M. & SRIRANGANATHAN, N. 2010. Brucellosis: a re-emerging zoonosis. *Veterinary Microbiology*, 140, 392-398. DOI: 10.1016/j.vetmic.2009.06.021.

STOLER, J. & AWANDARE, G.A. 2016. Febrile illness diagnostics and the malarial-industrial complex: a socio-environmental perspective. *BMC Infectious Diseases*, 16, 683. DOI: 10.1186/s12879-016-2025-x.

TALESKI, V., ZERVA, L., KANTARDJIEV, T., CVETNIC, Z., ERSKI-BILJIC, M., NIKOLOVSKI, B., BOSNJAKOVSKI, J., KATALINIC-JANKOVIC, V., PANTELIADOU, A., STOJKOSKI, S. & KIRANDZISKI, T. 2002. An overview of the epidemiology and epizootiology of brucellosis in selected countries of Central and Southeast Europe. *Veterinary Microbiology*, 90, 147-155. DOI: 10.1016/s0378-1135(02)00250-x.

WHATMORE, A.M., KOYLASS, M.S., MUCHOWSKI, J., EDWARDS-SMALLBONE, J., GOPAUL, K.K. & PERRETT L.L. 2016. Extended multilocus sequence analysis to describe the global population structure of the genus *Brucella*:

phylogeography and relationship to biovars. *Frontiers in Microbiology*, 7, 2049. DOI: 10.3389/fmicb.2016.02049.

WHO. 2005. Brucellosis in humans and animals. WHO guidance. In: L. HEYMANN (ed), Control of communicable diseases manual: an official report of the American Public Health Association, (World Health Organization/America Public Health Association, Washington DC).

WHO. 2006. *Brucellosis in humans and animals.* World Health Organisation in collaboration with the Food and Agricultural Organisation and the World Organisation for Animal Health. https://apps.who.int/iris/handle/10665/43597. *Accessed February 16, 2019.*

WHO. 2009. The control of neglected zoonotic diseases: a route to poverty alleviation. WHO, Geneva; 2009. https://www.who.int/neglected_diseases/zoonoses/9789241594301/en/. *Accessed February 16, 2019*.

Chapter 2: Literature Review

2.1. *Brucellae*

2.1.1. General

Brucellosis is a zoonosis that is caused by several closely related bacteria belonging to the genus *Brucella.* The genus is named after Sir David Bruce, who isolated the first species (now *Brucella melitensis*) in 1887 from a case of brucellosis which had killed a British soldier in Malta (Nicolleti, 1980). Clinical disease is known by different names in various parts of the world, including undulant fever, Malta fever, Bang's disease, contagious abortion, or infectious abortion.

2.1.2. Taxonomy of *Brucella*

The genus *Brucella (*Meyer and Shaw 1920) belongs to the family *Brucellaceae*, order Rhizobiales, class Alphaproteobacteria and phylum Proteobacteria (Bergey and Holt, 1994). Alphaproteobacteria include symbionts such as *Wolbachia and Sinorhizobium*, and facultative or obligate extracellular and intracellular pathogens of mammals such as *Bartonella*, Rickettsia and *Brucella* (Tsolis, 2002).

The *Brucella* genome is made up of two chromosomes (except *B. suis* biovar 3) and no plasmids. At present, the genus has twelve recognized species (http://www.bacterio.net/brucella.html) (Table 2.1). There is a high genomic similarity (> 90%) between *Brucella* spp. as demonstrated by molecular assays (Halling et al., 2005) which makes it difficult to distinguish strains based on basic molecular techniques (Huber et al., 2009). Previously, the genus *Brucella* was classified as one species (*B. melitensis)* with several biovars (Verger et al., 1985), but due to a lack of practicality of the classification system, the *Brucella* Taxonomy Subcommittee reverted to a classification system based on the six 'classical' species (*B. abortus, B. melitensis, B. suis, B. neotomae, B. canis, B. ovis*) (Osterman and Moriyón, 2006). This system distinguishes *Brucella* species based on microbiological, molecular, host preferences and pathogenicity differences (Osterman and Moriyón, 2006).

Table 2.1: *Brucella* species, their preferential hosts and zoonotic potential.

Species	Smooth /rough	Hosts
Brucella abortus	Smooth	cattle, camels, wild ungulates, humans
Brucella melitensis	Smooth	sheep, goats, cattle, camels, humans
Brucella ovis	rough	sheep, red deer (New Zealand)
Brucella suis	Smooth	pigs, wild boar, cattle, humans
Brucella canis	rough	dogs, humans
Brucella neotomae	Smooth	Wood/desert rats
Brucella ceti	Smooth	Dolphins, humans
Brucella inopinata	Smooth	Humans (breast implant)
Brucella microti	Smooth	Wild voles
Brucella papionis	Smooth	Baboons (*Papio* spp.)
Brucella pinnipedialis	Smooth	Seals, humans (rare)
Brucella vulpis	Smooth	Red foxes (*Vulpes vulpes*)

Sources: Corbel and Brinley-Morgan, 1984; Godfroid, 2002; Godfroid et al., 2005; Verger, 1985; Al Dahouk et al., 2017; Scholz et al., 2008; Foster et al., 2007; Scholz et al., 2009; Whatmore et al., 2014; Suárez-Esquivel et al., 2017

2.1.3. Smooth and rough strains of *Brucella*

Brucella species can also be classified into smooth (*B. abortus, B. melitensis, B. suis* and *B. neotomae*) and rough (*B. ovis* and *B. canis*) strains (Table 2.1) (FAO, 2003; Godfroid et al., 2004; Moreno and Moriyón, 2006) based on the structure of the lipopolysaccharide (LPS) component of the bacterial cell wall. The LPS of smooth strains consists of lipid A, a core of oligosaccharide, and the O-antigen, while the O-antigen is absent or is made up of a few sugars in rough strains (Corbel, 1997). The structure of the cell wall plays a role in the pathogenicity of strains, with smooth strains more pathogenic than rough strains (Jiménez de Bagües et al., 2004).

2.1.4. Morphology, growth characteristics and survival of *Brucella* spp.

Brucella are gram-negative, non-motile, aerobic cocci, coccobacilli or short rods that do not form spores or capsules. They are approximately 0.6 to 1.5 µm in length and 0.5 to 0.7 µm in width (Alton and Forsyth, 1996). Most *Brucella* species, except *B. melitensis,* grow best in an environment with 5 - 10% carbon dioxide (Alton et al., 1988). Growth of cultures is at maximum in media containing serum or blood, at a pH of 6.6 - 7.4 and temperatures of between 36 - 38^{o}C (Alton et al., 1988). The classical *Brucella* species are fastidious. They grow slowly in cultures due to limited metabolic activity, whereas *B. microti* and *B. inopinata* grow faster on cultures due to their high metabolic activity and biochemical profiles (Al Dahouk et al., 2010). On MacConkey agar*, Brucella* do not ferment lactose, or produce acid from glucose and do not show haemolysis on blood agar. Characteristically, they reduce nitrate, are catalase-positive, citrate-, indole- and VP-negative, and in most cases, oxidase and urease-positive (OIE, 2018).

2.1.5. *Brucella* as a cause of disease in animals and humans

Brucella melitensis, B. abortus, *B. canis* and *B. suis* are preferentially the main pathogenic species for sheep and goats, cattle, dogs, and pigs respectively. Bovine brucellosis caused by *B. melitensis* biovars has been reported infrequently in cattle raised together with small ruminants in Africa, Western Asia, and southern Europe (Verger, 1985; Jiménez de Bagües et al., 1991; Corbel, 1997; Godfroid et al., 2013). *Brucella suis* biovars have also been isolated from cases of bovine brucellosis (Szulowski et al., 2013; Ledwaba et al., 2014; 2019). Among the *Brucella* species, *B. melitensis* causes the most acute and severe form of undulant fever worldwide (WHO, 2006). *Brucella canis* is an underestimated cause of zoonotic disease (Dentinger et al., 2014). Few naturally acquired cases of *B. ceti* infections have been reported in humans (Sohn et al., 2003, McDonald et al., 2006), but reports of *B. ceti* and *B. pinnipedialis* as causes of human disease are rare (Osterman and Moriyon, 2006). *Brucella inopinata* is a zoonotic pathogen which was first isolated from a breast implant infection in humans (De et al., 2008).

Brucella abortus has eight biovars, 1–6, 9 (Whatmore, 2009) and 7 (Spickler, 2018); *B. melitensis* is divided into three biovars (1, 2, and 3) (Bricker and Halling, 1994);

while *B. suis* has five biovars (biovars 1, 2, and 3 infect pigs, biovars 4 and 5 infect reindeer and small rodents respectively) (Whatmore, 2009). In southern Africa, *B. abortus* biovar 1 has been isolated from most infections in cattle (Bishop et al., 1994; Mohan et al., 1996).

2.1.6. Survival of *Brucella* in different environments

Depending on environmental temperatures, *Brucella* bacteria can survive for longer periods in moist organic matter (Crawford et al., 1990; CFSPH, 2007) such as an aborted foetus kept under a shade (Crawford et al., 1990), but cannot withstand hot and dry conditions. Survival of *Brucella* in foods such as milk or cheese depends on moisture content, age of product, temperature, pH, presence, or absence of other bacteria. A pH of less than 3.5, as in ripened cheese, kills *Brucella* (WHO, 2006). Muscle tissue contains low numbers of bacteria than lymph nodes, uterine fluids and the infected foetus (WHO, 2006). Survival of the bacteria in chilled meat is of limited duration because of the low pH attained by matured meat. However, *Brucella* can survive for many years in frozen meat (EC, 2001).

2.2. Epidemiology of brucellosis

2.2.1. Global distribution and economic importance

Brucellosis is a neglected zoonosis (Pappas, 2010) that is emerging and re-emerging in many parts of the world (WHO, 2011). The disease is endemic and a public health concern in many parts of the world (Moreno, 2014) including Africa (Ducrotoy et al., 2017), the Middle East (Corbel, 1997), Mediterranean region (Pappas et al., 2006), Asia, Central and Southern America (Pappas et al., 2006). Brucellosis has been reported in 86 different countries worldwide (Khan and Zahoor, 2018), but several countries including Japan, New Zealand, Australia, Canada, and Western and Northern Europe have eradicated the disease (OIE, 2018). In sub-Saharan Africa, the disease is endemic in countries or parts of countries where extensive pastoral production systems are practised and where disease surveillance and control activities are limited (McDermott and Arimi, 2002). In Namibia, serological evidence has shown that the disease is endemic in cattle, sheep, and goats (Magwedere et al., 2011; Madzingira and McCrindle, 2014; 2015; 2016).

Brucellosis places large burdens on human healthcare systems and limits the economic potential of animals, individuals, communities, and nations (Franc et al., 2018). The economic burden of brucellosis on cattle production are due to abortions, retained placentas, temporary or permanent infertility in both cows and bulls, birth of weak calves that die soon after birth, arthritis or bursitis, reduced meat and milk yield, loss of infected animals due to culling or slaughter, costs associated with implementing control or prevention programs (McDermott and Arimi, 2002; Corbel, 2006; McDermott et al., 2013; Franc et al., 2018). In addition, brucellosis poses a potential barrier to national and international trade due to the strict and costly sanitary measures that are implemented to control the disease (Bricker and Halling, 1994; Sreevatsan et al., 2000; Godfroid, 2002; Caminiti et al., 2017). Financial losses attributable to bovine brucellosis outbreaks and control have been estimated at US$ 448 million in Brazil (Santos et al., 2013), US$ 3.4 billion in India (Singh et al., 2015) and US$150 million in the United States of America (Boschiroli et al., 2001) per year.

2.2.2. *Brucella* transmission

2.2.2.1. Transmission in cattle

Transmission of brucellosis is primarily by ingestion, inhalation or contact with *Brucella* bacteria that are present in large numbers in contaminated milk, feed, or water, foetal membranes, aborted foetuses and post parturient uterine or vaginal discharges of infected animals (Garin-Bastuji et al., 1998; Mangen et al., 2002). Inhalation of contaminated aerosol or dust particles occurs when cattle sniff at aborted fetuses, fetal membranes and vaginal discharges (Godfroid et al., 2004). *Brucella* can also enter a host through direct contact with mucus membranes, wounds, intact or broken skin (Ibironke et al., 2008; Kahn, 2008). Vertical transmission affects a limited number of calves born of dams with latent infections. Up to 10% of calves born to *B. abortus* infected cows have latent infections and remain seronegative until about the middle of the first gestation or later, when antibodies become detectable or abortions occur (Catlin and Sheehan, 1986). Some calves infected in utero or at birth may clear the infection after some months (Nicoletti, 1980). The majority of latently infected female calves abort at their first

pregnancy (Godfroid, 2018) and transmit the infection to the rest of the herd. Suckling of colostrum or milk from infected dams can result in neonatal infection (Bercovich et al., 1990). In particular, the practice of mixing colostrum from different cows for calf feeding increases the risk of infection (FAO, 2006). Persistent infection of the mammary glands and supramammary lymph nodes can lead to constant or intermittent shedding of the organisms in the milk in subsequent lactations. *Brucella* infection of the bovine mammary gland may also occur through the teats from contaminated milk cups (Spickler, 2018). *Brucella* infected cows abort once in their lifetime but may carry infection for many years or for life (Spickler, 2018), with subsequent pregnancies carried to term and excretion of bacteria persisting in vaginal discharges and milk.

In bulls, infection may be acquired in-utero or in the early calf hood period, localize and persist in the testes and accessory sex glands, with shedding of *Brucella* in semen and urine (Spickler, 2018). Although bulls may intermittently excrete *Brucella* bacteria in semen, venereal transmission is not considered an important source of transmission to cows (Godfroid, 2018), unless the infected semen is deposited in the uterus by artificial insemination (Amin et al., 2001; FAO, 2006). It has been hypothesised that antimicrobial factors in the cervix inhibit *Brucella* survival (Nicoletti, 1980), thus limiting the chances of infection. Where recommended procedures for embryo harvesting, preservation and transfer have been followed, embryo transfer is safe in all species (WHO, 2006).

2.2.2.2. Transmission to humans

Humans are accidental and dead-end hosts of brucellosis (Ibironke et al., 2008). The disease is an occupational risk for professions such as herders, veterinarians, abattoir workers, dairy workers, livestock farmers, laboratory professionals, tanners and hunters who work with and handle animals and their products (EU, 2001; Godfroid, 2002; WHO, 2005). Infection is acquired from animal reservoirs directly or indirectly through contact with infected animals or animal material such as aborted foetuses, calves born to infected cows, gynaecological and obstetrical manipulations, rectal examination of infected cows, vaginal discharges, foetal membranes, foetal fluids, or the ingestion of contaminated animal products (raw milk, meat, home-made

cheese and ice cream) or the inhalation of contaminated animal faeces (Peelman and Dekeyser, 1987; Corbel, 1997; Tuon et al., 2017). Lambing, kidding, and parturition are periods associated with a high risk of human infection (EU, 2001). Consumption of contaminated unpasteurized milk and dairy products is the most common and epidemiologically important source of human infection (Godfroid et al., 2005) because large volumes can be consumed at one time (FAO, 2010). Among dairy products, cheese made from raw milk presents a greater risk for human infection (Carvalho Neta et al., 2010) because the cheese making process concentrates *Brucella* bacteria (EU, 2001). Soft fresh cheeses present a greater risk for human infection than matured hard cheese, because the higher pH promotes *Brucella* survival. In communities, where the liver and spleen are consumed raw, a risk of *Brucella* infection exists, as these organs can be highly contaminated (Godfroid et al., 2005). Inhalation of *Brucella* is an important route of infection for persons working in laboratories and abattoirs (Seleem et al., 2010). Very rarely, person-to-person transmission through tissue transplantation, blood transfusion, bone marrow transplantation, hospital exposure, sexual or laboratory transmission, transplacental or perinatal exposure may occur (Giannacopoulos et al., 2002; Carrera et al., 2006; Kato et al., 2007; Kotton, 2007; Mesner et al., 2007; Tuon et al., 2017).

2.2.3. Risk factors for infection in cattle

Risk factors associated with *Brucella* infection can be categorized into those determinants necessary for the transmission and maintenance of the disease within herds (herd immunity, type of housing, stocking density, use of maternity pens) and those factors that are required for the transmission of the disease between herds (lack of biosecurity, intermingling with other herds and sources of water) (Nicoletti, 1980). Factors related to the host, the agent, the environment, and management practices determine the extent of exposure, spread and maintenance of brucellosis in a geographical area (Godfroid, 2002). Risk factors for the transmission or occurrence of brucellosis in domestic animals have been reviewed by Coelho et al. (2015) and include absence of designated calving areas, age, sex, breed, species, herd size, availability of cleaning and disinfection procedures, use of communal pastures, contact with wild animals, intensive management, introduction of new

animals into a herd and stocking rate. A study in Cameron also identified age, sex, knowledge of brucellosis, herd size and geographical location as animal-level risk factors for brucellosis (Awah-Ndukum et al., 2018).

Host susceptibility and establishment of *Brucella* infection is variable and dependent on the ruminant species, reproductive status, age, sex, immune status, virulence, and the infection dose (EU, 2001). Pregnant cows are at a higher risk of *Brucella* infection than non-pregnant cattle and bulls (Crawford et al., 1990), due to the tropism of *Brucella* for the gravid uterus and foetal tissues (Coelho et al., 2013). The gravid uterus produces erythritol (FAO, 2006), a sugar that promotes the growth of some *Brucella* bacteria (Crawford et al., 1990). However, pathogenic *Brucella* species have also been recovered from the reproductive tracts of cattle in the absence of erythritol (EU, 2001), putting doubts to this theory. *Brucella abortus* strain 19 is inhibited by the presence of erythritol (Duffield et al., 1984). However, some variants of S19 can tolerate erythritol, which may be the reason why S19 vaccines can occasionally cause abortions in cattle (Dorneles et al., 2015).

Seroprevalence of brucellosis has been reported to be higher in older than younger cattle (Muma et al., 2006; Solorio-Rivera et al., 2007; Sanogo et al., 2012) due to an age-related waning of immunity and a longer period of exposure to the pathogen (Megersa et al., 2011). Since susceptibility increases with sexual maturity and in pregnant cattle, brucellosis is considered a disease of adult cattle (Bekele et al., 2011). Therefore, cows that remain for long periods in a herd present a higher risk of infection to the herd.

Abortion history, herd size, insemination method and farm management practices (lack of disinfection of environment after abortion, sharing of calving space, new animal purchases and sharing of grazing by animals from different herds) have been identified as risk factors for the occurrence of brucellosis in a herd (Makita et al., 2011; Anka et al., 2014; Lindahl et al., 2014). The major risk factor for introducing brucellosis in a free cattle herd is the introduction of new animals without implementing biosecurity measures (EC, 2001; McDermott and Arimi, 2002). In Zambia, geographical location, husbandry practices, herd size, breed and contact

with wild animal species have been reported as risk factors for brucellosis in cattle (Muma et al., 2007). The development of the game farming industry has also been implicated as a cause of the re-emergence of brucellosis in livestock, because of the lack of pre-movement screening and an increase in the density of infected game species (Godfroid, 2002). Mixed rearing of several animal species has been identified as a risk factor for *Brucella* seropositivity due to the presence multiple sources of infection (Al-Majali et al., 2008; Bekele et al., 2011; Megersa et al., 2011).

Large and mobile pastoral herds that regularly mingle with other herds at communal grazing areas (EU, 2001; McDermott and Arimi, 2002; WHO, 2006) in southern Africa, as also happens in Namibia, are at a greater risk of brucellosis than confined herds. This explains the higher brucellosis seroprevalence reported in cattle in communal areas than in urban and peri-urban areas (Boukary et al., 2011; 2013). A higher prevalence of brucellosis is associated with large than small cattle herds (Coelho et al., 2013). In small cattle herds, diseased animals can be easily identified and a high vaccination coverage can be easily achieved (Mainar-Jaime and Vázquez-Boland, 1999; Godfroid et al., 2011). The time of parturition is also a period of high risk of infection for cattle, sheep, goats, and humans (WHO, 2006).

2.2.4. Prevalence of brucellosis in cattle

McDermott et al. (2013) reported a prevalence of 0-68% in cattle in Africa and Asia based on different methods. In South Africa, the prevalence of bovine brucellosis has been estimated at 0-1.5% in cattle herds (Hesterberg et al., 2008) and at 5.5% at an abattoir (Kolo et al., 2019). In Namibia, results of a few serosurveys that have been carried out over the years, show that the prevalence of anti-*Brucella* antibodies in domestic ruminants including cattle, is less than 2% (Magwedere et al., 2011; Madzingira and McCrindle, 2014; 2015; 2016; Madzingira et al., 2020). In Argentina, herd and individual animal prevalence are around 10–15% and 4–5% respectively, in Chile and Uruguay, the prevalence of brucellosis is less than 1% (Aznar et al., 2014), while in Central America, prevalence of bovine brucellosis is estimated at 4-8% (Lucero et al., 2008).

2.2.5. Prevalence, incidence, and risk factors for brucellosis in humans

Prevalence and spatial distribution of brucellosis in the human population typically corresponds with the observed trends in animals (Corbel, 2006; Makita et al., 2010; Osoro et al., 2015). The highest prevalence of human disease has been reported in Africa, Asia, Latin America**,** and the Middle East (Hull and Schumaker, 2018). Ineffective animal and public health programs have been implicated as the reason for increasing brucellosis cases in humans (FSAI, 2009). In high-risk human populations, such as veterinarians, livestock handlers, and abattoir workers, an average prevalence of 11% has been reported, while in hospital patients in which the disease was consistent with the clinical picture, a seroprevalence of 7% was reported (McDermott et al., 2013). About 12% of laboratory workers in Spain get brucellosis during routine fieldwork (Bouza et al., 2005; Kose et al., 2014). In the Czech Republic, in a survey of 479 veterinarians, 32.4% were serologically positive, whilst 17.5% showed clinical symptoms of brucellosis (Kouba, 2003). A higher prevalence of brucellosis has been reported in males (90%) (Tay et al., 2015) due to their regular involvement in the handling of livestock and their products (Khan and Zahoor, 2018). However, other studies have reported a higher incidence rate of the disease in females in rural areas (Boschiroli et al., 2001; Mantur et al., 2006) further asserting the fact that brucellosis has no gender association but is occupation specific. Reports indicate that brucellosis is common in people of 13-45 years of age (Makita et al., 2008; Tay et al., 2015), but the aged are more vulnerable to severe forms of the disease (Fallatah et al., 2005). The disease is rarely reported in children except in regions where pasteurization of milk is not practiced (Alp and Doganay, 2008). Among other factors, the socio-economic status of a population (Musallam et al., 2016), age (Fallatah et al., 2005; Makita et al., 2008) and the occurrence of human immuno-deficiency virus (HIV) infections (Moreno et al., 1998) have been identified as factors affecting the incidence of the disease in endemic areas.

Incidence of human brucellosis varies widely. About half a million cases of human brucellosis are reported worldwide each year (Pappas et al., 2006; Pappas, 2010). True incidence is estimated to be 10 - 25 times higher (WHO, 1997; Berger, 2016), with most cases occurring in developing countries (Hald et al., 2016). The highest incidence of human brucellosis around the world has been reported in the Middle

East (Pappas et al., 2006). Globally, the incidence of the disease has been reported to vary from <0.03 to >160 per 100 000 human population with the highest incidence in Syria (Pappas et al., 2006; Spickler, 2018). In most developed countries where brucellosis has been eradicated from animals, incidence is less than one case per 100,000 population. However, in the EU and the USA, an annual incidence of brucellosis of 32.5 (Minas et al., 2007a) and 0.02 to 0.09 per 100 000 population (Dean et al., 2012a) respectively, has been reported. In Africa, incidence of <0.09 to >8.43 per 100 000 population has been reported (Taleski et al., 2002; Pappas et al., 2006; Seleem et al., 2010), while in South Africa, the annual incidence rate was between <0.1 and 0.3 per 100,000 population (Küstner, 1985). Official reports on incidence, prevalence, or risk factors for human brucellosis in Namibia were not found in the literature.

2.3. Clinical manifestation

2.3.1. Cattle

The incubation period for brucellosis varies from 14 to 120 days (Seifert, 1996). Brucellosis manifests acute to sub-acute or chronic signs depending on the organ and the type of animal infected (Currò et al., 2012). Ruminants infected for the first time generally abort once in their lifetime in the mid third or second half of gestation (5^{th} to 9^{th} month), with subsequent pregnancies carried to term (Radostits et al., 2000; CFSPH, 2007; Carvalho Neta et al., 2010) and in milk (OIE, 2018). Depending on the severity of uterine infection, stillbirths, retained placentas and the birth of weak and sickly newborns that may die soon after birth may occur (Spickler, 2018). Retention of the placenta and secondary metritis are common sequelae to abortions (Walker, 1999) which may exacerbate reproductive wastage by delaying the return to oestrus and may produce permanent infertility. Other signs that have been observed in cattle are delayed calving, male infertility, and a marked reduction in milk yield (Garofolo et al., 2016; Celebi et al., 2007; Arif et al., 2017; Currò et al., 2012) with no apparent clinical evidence of infection in the udder (Spickler, 2018). Pregnant cows may show visible swelling of the mammary gland which extends to the navel region (Khan and Zahoor, 2018). Bleeding from the vagina may also occur, even if the cow does not abort (Fensterbank, 1987). In bulls, the disease may in rare cases be characterized by fever, unilateral or bilateral orchitis with enlarged or normal testes

(Spickler, 2018). Unilateral or bilateral arthritis and hygromas can develop in long-term infections and cause a negative impact on production and reproduction (Alton, 1990; Mangen et al., 2002; CFSPH, 2007; OIE, 2018).

2.3.2. Humans

Exposure to *Brucella* may or may not result in infection or antibodies detectable by serology and may not always result in clinical symptoms (Godfroid, 2017). Spontaneous recovery may occur following infection. Human brucellosis is an acute febrile flu-like disease with varied and non-specific symptoms that are often confused with symptoms due to other infectious diseases such as malaria and typhoid, paratyphoid and influenza (Seleem et al., 2010). After an incubation period of 2 to 4 weeks or several months long, the disease manifests as an acute infection accompanied consistently by an intermittent or undulant fever, with body temperature reaching up to 40^{o}C (Reguera et al., 2003; WHO, 2005; FAO, 2006; Seleem et al., 2010). Symptoms may go on for months before the illness becomes severe and debilitating as to require medical attention (FAO/WHO, 1986). The course of the disease may progress to subacute or a chronic relapsing infection with severe complications (Pappas et al., 2005; Dean et al., 2012b), if diagnosis and treatment are delayed (FAO, 2006). Clinically, infected patients may exhibit a cough, experience difficult breathing (Sharda and Lubani, 1986; Mili et al., 1993; Pappas et al., 2005), malaise, anorexia, headache, arthralgia, myalgia, chills, sweating, chronic fatigue, insomnia, anorexia, weight loss, polyarthritis, meningitis, pneumonia, endocarditis, back pain and prostration (Araj, 1999; Reguera et al., 2003; Godfroid, 2002; Carvalho Neta et al., 2010; Seleem et al., 2010; Buzgan et al., 2010; Dagli et al., 2011; Zhong et al., 2013). The severe complications related to the musculoskeletal system such as joint and back pain are linked to the chronic phase of the disease (Kose et al., 2014). In pregnant women, abortions in the first or second trimesters, congenital malformations, premature delivery, death of the foetus, newborn or even maternal death may occur (FAO, 2006; Seleem et al., 2010; Vilchez et al., 2015). In males, orchitis with visible swelling of the testes and burning micturition due to urethritis have been reported in infected individuals (Smith and Kadri, 2005). Despite the severe clinical manifestations and the life-threatening

complications, the case fatality rate for untreated cases of brucellosis is extremely low (<1%) (Al Dahouk and Nöckler, 2011).

2.4. Diagnosis of brucellosis

2.4.1. Bovine brucellosis

Brucellosis should be considered in bovines presenting with a suggestive clinical picture using laboratory tests. Diagnostic tests for the detection of *Brucella* infection have been extensively reviewed (EU, 2001; Nielsen, 2002; Gall and Nielsen, 2004; Nielsen et al., 2004; FAO, 2006; Godfroid et al., 2010; Nielsen and Yu, 2010; OIE, 2018). Diagnosis of bovine brucellosis is based on a battery of diagnostic tests since no one assay can identify all infected and disease-free animals.

2.4.1.1. Serological diagnosis of bovine brucellosis

Indirect diagnostic tests based on serology are the most common and readily available approaches for detecting *Brucella* infections in bovines (Weynants et al., 1995). Test repetitions and combinations are often necessary to reach a diagnosis. Serological tests are performed for the purposes of screening, prevalence studies, confirmatory diagnosis, certification for trade purposes, or for continuous surveillance following eradication of the disease. An ideal serological test is one that is validated and able to differentiate between infected and exposed animals (Blasco et al., 1994). Most serological tests cannot differentiate between antibodies induced by vaccination with smooth *Brucella* spp. vaccines and antibodies produced by other gram-negative bacteria with an identical OPS structure such as *Yersinia enterocolitica* O: 9 (Nielsen et al., 2006; McGiven et al., 2012), which leads to false positive serological results (Weynants et al., 1996; Muñoz et al., 2005; Corbel, 2006; OIE, 2018). *Escherichia coli* O157:H7, *Vibrio cholera, Francisella tularensis* can also cross react with *Brucella* spp. in serological tests due to the shared antigenic determinant N-acetylated-D-perosamine (Garin-Bastuji et al., 1999). Serological tests cannot detect *Brucella* antibodies during the early stages of the infection (up to 12–16 days). Furthermore, all naturally occurring biovars of *Brucella* spp. have common immunodominant epitopes associated with the surface smooth LPS (sLPS), therefore the infecting species cannot be identified using serological tests (Godfroid et al., 2010; Ducrotoy et al., 2016).

In Namibia, serological testing of brucellosis is carried out using the Rose Bengal test (RBT) as a screening test and the complement fixation test (CFT) as a confirmatory test following the latest guidelines in the OIE (2018).

2.4.1.1.1. Rose Bengal test (RBT)

The RBT is a fast and simple slide agglutination test that is performed using a stained *B. abortus* antigen suspension at a buffered pH of 3.6-3.7 and plain test serum. It is based on the reactivity of antibodies in test serum against the stained antigen. It is a highly sensitive test, which makes it an ideal test for the initial screening of herds for infection (OIE, 2018). However, RBT has a low specificity especially in low prevalence areas (FAO, 2010). Ruiz-Mesa et al. (2005) reported an overall sensitivity of 92.9% for the RBT. According to Bercovich (1998) and Diaz-Aparicio et al. (1994), the RBT has a specificity (*Sp*) of between 71-80% and a sensitivity (*Sp*) of 78-100%. Other studies (Gall and Nielsen, 2004; Muñoz et al., 2005; Minas et al., 2007b; Ramirez-Pfeiffer et al., 2008; Greiner et al., 2009; Matope et al., 2011; Abernethy et al., 2012; Sanogo et al., 2013) have reported *Se* and *Sp* in cattle of 53% to 100% and 79% to 100% respectively. False negative results are rare, but false positives are possible, especially in cattle vaccinated with *B. abortus* S19.

2.4.1.1.2. Complement fixation test (CFT)

The CFT is a more specific test than the RBT, but is time consuming and complex, requiring several reagents, specialised equipment, and skilled laboratory personnel. It is commonly used in series (warm fixation) to confirm the status of animals reacting positive on the RBT (OIE, 2018). In other regions, ELISA and FPA assays are increasingly being preferred for this purpose (OIE, 2018). Haemolysed serum samples or serum with anti-complement activity can limit the performance of the CFT (Nielsen et al., 2004). The CFT detects IgM and IgG anti-*Brucella* antibodies that activate complement. A sensitivity of 53-100% and a specificity of 65-100% has been reported for the CFT (Gall and Nielsen, 2004; Greiner et al., 2009; Abernethy et al., 2012). Bercovich (1998) determined a specificity of 98% and a sensitivity of 81% for the CFT. Like other serological assays, CFT may give positive results in cattle that were vaccinated using *B. abortus* S19 vaccine. The relatively low sensitivities of the RBT and CFT tests may result in discrepancies between results of the two tests. Parallel use of the RBT and CFT tests has been reported to increase the sensitivity of the diagnosis compared to series application, but is more expensive (FAO, 2010). The RBT and CFT are OIE recommended tests for international trade in livestock (EU, 2001; Nielsen, 2002; FAO, 2010).

2.4.1.1.3. Enzyme linked immunosorbent assay (ELISA)

ELISA tests detect IgM, IgG and IgA antibodies against the smooth LPS allowing for the detection of the stage of *Brucella* infection (acute or chronic). It is an ideal assay for screening large populations of cattle for *Brucella* antibodies especially in endemic areas. It is recognized that the detection of IgG antibodies (as by ELISA) is a more sensitive approach for diagnosing brucellosis than the detection of IgM antibodies. However, a combination of IgM and IgG ELISA tests is recommended as the most effective for the diagnosis of brucellosis (Araj, 2010; Sathyanarayan et al., 2011; Agasthya et al., 2012). In cattle, indirect ELISA (i-ELISA) is used to detect anti-*Brucella* antibodies in serum or milk (Di Febo et al., 2012). It is sensitive and specific for *B. abortus* or *B. melitensis* but cannot differentiate antibodies induced by *Brucella abortus* S19 or *Brucella melitensis* Rev1 vaccine strains (OIE, 2018). The sensitivity of i-ELISA has been reported to vary from 96% to 100% and the specificity from

93.8% and 100% (Gall and Nielsen, 2004). In general, the diagnostic sensitivity of i-ELISA is equal or greater than that of the RBT or the CFT, but the specificity is lower (Praud et al., 2012).

Competitive ELISA (c-ELISA) is another ELISA assay variant. It uses smooth *Brucella* S-LPS or OPS antigens to detect anti-*Brucella* antibodies in cattle, sheep, goats, and pig sera. The c-ELISA can detect and differentiate antibodies resulting from vaccination and natural infection (Nielsen et al., 1996; Nielsen and Yu, 2010). It has also been reported to reduce false positive serological reactions caused by cross-reacting bacteria in cattle (Muňoz et al., 2005). The sensitivity of the assay is between 92% and 100%, while the specificity ranges from 90% to 99% (Perrett et al., 2010; Godfroid et al., 2010). In most cases, results of ELISA and CFT tests are compatible (Ruppanner and Taaijke, 1980; Stemshorn et al., 1980; Bercovich and Taaijke, 1990). However, because ELISA is more expensive than CFT, most developing countries use CFT rather than ELISA as a confirmatory test, despite CFT being a laborious method.

2.4.1.1.4. Other serological tests

The milk ring test (MRT) is a cheap, simple, and fast assay for screening for brucellosis in dairy herds by testing combined milk samples (Radostits et al., 2000). It detects lacteal anti-*Brucella* IgM and IgA antibodies bound to milk fat globules (OIE, 2018). As a result, milk that contains a low concentration of lacteal IgM and IgA or that lacks the fat-clustering factors may give false negative results on the test (Patterson and Deyoe, 1976). Lacteal antibodies decline rapidly following an abortion or parturition, thus reducing the reliability of the MRT as a test for detecting anti-*Brucella* antibodies in bulk milk or individual cows. In large herds (>100 lactating animals), the sensitivity of the test is low (Radostits et al., 2000). False positive reactions may occur in animals vaccinated about four months prior to testing; in milk at the end of a lactation and in samples containing abnormally acidic milk due to colostrum or mastitis (OIE, 2018). Therefore, the MRT is not recommended for small dairy herds, where colostrum and mastitis can have a greater impact on the test results.

The fluorescence polarization assay (FPA) is a rapid and simple but less common assay, which quantifies antibody and antigen reactions. The assay has a high sensitivity and specificity in cattle and goats (Lucero et al., 2003, McGiven et al., 2003, Ramirez-Pfeiffer et al., 2006). FPA can be performed in field settings but requires specialised equipment that is expensive.

The brucellin skin test (BST) is an immunological assay that can be used to screen unvaccinated cattle herds for brucellosis (Pouillot et al., 1997). However, cattle vaccinated with *B. abortus* S19 or RB51 may react positively on this test for many years (Pouillot et al., 1997; De Massis et al., 2005). Therefore, the BST is not useful in areas where cattle are routinely vaccinated against brucellosis. A positive result shows as a local swelling or skin thickening of 1.5–2 mm (OIE, 2018).

2.4.1.2. Culture, isolation, and identification of *Brucella* from specimens

Culture, isolation and typing of *Brucella* mainly from tissues (animals), blood, bone marrow and cerebrospinal fluid (humans) is the gold standard test for confirmation of *Brucella* infection (Nielsen, 2002). In cattle, lymph nodes, spleen, foetal membranes, aborted foetuses, vaginal discharges, hygroma fluid, milk including colostrum, epididymis and testes are valuable samples for culture and isolation of *Brucella* bacteria (FAO, 2003; FAO, 2010; OIE, 2018). Bacterial isolation requires a well-equipped high biosecurity laboratory (minimum of a BSL 2+), skilled personnel and presents an occupational health hazard to laboratory staff (Godfroid et al., 2014). Culture has low sensitivity, especially in chronic cases where bacterial numbers are low (Godfroid et al., 2004) and is impractical for surveillance testing of large herds. Often, several specimens from the same sample must be tested to increase sensitivity (Gall and Nielsen, 2004). Further, bacterial isolation has a relatively long culture time due to the slow growing nature of smooth *Brucella* species and characterization and typing tests are complex (Mangen et al., 2002; FAO, 2010; Godfroid et al., 2010). However, bacterial isolation enables speciation, typing and determination of antibiotic susceptibility profiles of isolates and epidemiological investigation of disease outbreaks to be carried out (Lee et al., 2013).

Commercial basal solid media such as tryptose soy agar (TSA), blood agar and serum dextrose agar (SDA) supplemented or not supplemented with equine or bovine serum (2-5%) are ideal media for *Brucella* growth and observation of colonial morphology (Alton et al., 1988). For contaminated field specimens such as lymph nodes, the use of selective media, such as Modified Farrell's medium (FM) that inhibit the growth of contaminants is highly recommended (OIE, 2018). Modified Thayer-Martin (mTM) media is a less selective media and opaque, hence, it is not suitable for observation of colonial morphology (Alton et al., 1988). Both Farrell's and the Thayer-Martin media are suitable for the culture and isolation of *Brucella* from homogenized tissue samples (Godfroid et al., 2010). CITA, is a selective media that is translucent and more sensitive than mTM and FM for isolating smooth *Brucella* species (including *B. abortus*) from contaminated field specimens (De Miguel et al., 2011). *Brucella* cultures are grown for 10-14 days in an atmosphere containing 5-10% carbon dioxide (except *B. melitensis*), at temperatures of 36 - 38^{o}C and a pH of 6.6-7.4 (Alton et al., 1988).

2.4.1.2.1. Identification of *Brucella* from cultures

Presumptive identification of *Brucella* spp. from cultures is performed by a combination of assessment of colonial morphology, Gram or Stamp staining, growth characteristics, catalase, oxidase, and urease tests (Alton et al., 1988; Corbel and Banai, 2005) and the slide agglutination test. In the slide agglutination test, polyclonal antiserum against smooth *Brucella* spp. is mixed with a suspension of colonies in saline (Alton et al., 1988; Al Dahouk et al., 2003). Smooth *Brucella* colonies are round, translucent, with a pale honey colour, a diameter of about 1-2 mm, and a convex, circular, and smooth outline (OIE, 2018). Rough colonies are slightly opaque, with a granular surface (Poester et al., 2010).

Microscopic examination of Stamp (modified Ziehl-Neelsen) stained smears of pathological specimens such as vaginal discharges, placental tissue or abomasal content from aborted foetuses gives a presumptive identification (Alton et al., 1988). *Brucella* spp. bacteria appear singly, in pairs or in clumps of gram-negative bacteria that stain red against a blue background (FAO, 2003; Godfroid et al., 2004). Morphologically, they appear as short rods, cocci or coccobacilli, measuring about

0.6 µm to 1.5 µm long and 0.5 µm to 0.7 µm wide (Alton and Forsyth, 1996). However, Stamp's staining method can give false positive results in the presence of other abortion pathogens such as *Chlamydophila abortus* and *Coxiella burnetii* that have similar morphology and staining properties (Alton et al., 1988). In Namibia, the Stamp staining method is the main test for presumptive diagnosis of brucellosis on pathology specimens, due to the absence of a functioning BSL 2+ laboratory.

Further identification of *Brucella* colonies from cultures can be performed using biochemical tests (urease and oxidase tests) and the agglutination test on a slide with polyclonal anti-*Brucella* serum (OIE, 2018).

Phenotypic methods for the identification of species and biovars (biotyping) are described by Alton et al. (1988). Biotyping of an isolate requires the association of cultural, morphological, biochemical, serological and phage-lysis characteristics (OIE, 2018). However, phage lysis and agglutination with anti-A, -M, or -R are specialised tests which are best carried out by internationally accredited reference laboratories. Serological, biochemical or phage-lysis characteristics of *Brucella* should be established from colonies that have been verified as being perfectly smooth. The following are the classical tests used for biotyping as described by Alton et al. (1988) and the OIE (2018):

- phage lysis using a phages such as Weybridge (Wb), Tbilisi (Tb), Izatnagar1 (Iz1) and R/C and agglutination tests using anti-A, -M or -R monospecific sera,
- test for dependence on carbon dioxide for growth that is performed on primary cultures,
- test for production of hydrogen sulphide (H_2S) using lead acetate papers and
- growth in the presence of basic fuchsin and thionine dyes.

2.4.1.3. Polymerase chain reaction (PCR) based techniques

Due to the drawbacks associated with serological tests and conventional biotyping tests, methods that are based on the detection of *Brucella* specific DNA have become popular in many laboratories worldwide. For *Brucella* diagnosis, molecular techniques are more specific, reliable, and safe for laboratory personnel (Castano

and Solera, 2009). The best molecular assays for the identification of *Brucella* spp. are those that are targeted at specific genes that are common to *Brucella* spp. such as the 16S-23S interspacer (ITS) region (Hillis and Dixon, 1991), the *IS*711 insertion sequence (Halling et al., 1993), the *bcsp31* gene encoding a 31-KDa protein (Mayfield et al., 1988), *omp2* gene (Leal-Klevezas et al., 1995) or the *per* gene (Bogdanovich et al., 2004). Polymerase chain reaction (PCR) assays and associated molecular methods such as restriction fragment length polymorphism (RFLP), provide an alternative means for detecting and confirming *Brucella* species and some biovars causing an infection (Bricker, 2002; Moreno et al., 2002; Ocampo-Sosa et al., 2005; Godfroid et al., 2010; López–Goñi et al., 2011; Whatmore and Gopaul, 2011). PCR methods for the diagnosis of *Brucella* have been described by Bricker (2002). PCR has a high sensitivity and specificity, which is useful for detecting small numbers of *Brucella* (dead or alive) especially in cases where antibiotic treatment has been initiated. The assay can be used to detect *Brucella* DNA extracted from clinical specimens such as serum, whole blood, urine, cerebrospinal fluid, synovial or pleural fluid, pus, and tissues (Queipo-Ortuno et al., 2008; Wang et al., 2014). PCR based methods can distinguish with a high degree of accuracy, between acute, subacute, and chronic *Brucella* infections (O'Leary et al., 2006). However, accuracy of detection of *Brucella* DNA can be limited by the stage of infection and location of *Brucella* bacteria at the time of sampling (O'Leary et al., 2006). Conventional PCR using *Brucella* genus specific primers, is the only molecular assay that is used to complement the diagnosis of brucellosis in animal tissues in Namibia.

Several multiplex PCRs that make use of a variety of primer combinations have been developed and validated to identify *Brucella* at the species level. The AMOS (*abortus, melitensis, ovis, suis*) PCR is one such multiplex PCR. The assay can identify *B. abortus* (bv. 1, 2 and 4), *B. melitensis*, *B. ovis*, *B. suis* bv. 1, *B. abortus* vaccine strains S19 and RB51 based on IS711 insertion element (Bricker and Halling, 1995; Ocampo–Sosa et al., 2005). AMOS-PCR is also an ideal assay for the identification of *Brucella* species from cultures. The Bruce-ladder multiplex PCR can identify and differentiate between all the *Brucella* species and vaccine strains *B. abortus* strain 19 (S19), *B. abortus* RB51 and *B. melitensis* Rev. 1 in a single test

(López-Goñi et al., 2008). Unlike other PCR assays, Bruce-ladder can also detect *B. abortus* biovars 3, 5, 6, 9; *B. suis* biovars 2, 3, 4, 5; *B. neotomae, B. pinnipedialis* and *B. ceti* DNA (OIE, 2018).

Brucella isolates can be further characterized using Multi-locus Variable Number Tandem-Repeat (VNTR) Analysis (MLVA) and/or Whole Genome Sequencing-Single Nucleotide Polymorphism (WGS-SNP) analysis. MLVA enables in-depth epidemiological studies of *Brucella* (Al Dahouk et al., 2007a; Whatmore et al., 2016; Ducrotoy et al., 2017). MLVA and WGS-SNP assays can identify and differentiate *Brucella* at the species, biovar and at the individual strain level. MLVA analyses the variability of loci presenting repeated sequences (Le Flèche et al., 2006; Whatmore, 2009) and generates data that can be used to analyse genetic diversity among isolates in a disease outbreak situation and in evolutionary studies (Whatmore et al., 2006; Ferreira et al., 2012; Garofolo, et al., 2013) to determine the origin and geographical distribution of an infection (Whatmore et al., 2016). In other words, MLVA allows tracing back to the source of infection in countries in which several biotypes are circulating. When one specific biovar is most frequently isolated in a country or region, MLVA and Multi Locus Sequence Analysis (MLSA) are ideal for typing and fingerprinting of isolates for epidemiological purposes (Le Flèche et al., 2006).

WGS involves the characterization of the *Brucella* genome and is crucial for identifying virulence factors among *Brucella* species (Wattam et al., 2014). Whole genome sequences of *B. abortus* (Halling et al., 2005), *B. suis* (Paulsen et al., 2002), and *B. melitensis* (DelVecchio et al., 2002) have been elaborated using this method. A combination of WGS and SNP can reveal genetic diversity within *Brucella* species. It performs better than the MLVA and multiplex PCRs (Ledwaba et al., 2019).

2.4.2. Diagnosis of brucellosis in humans

Detection of brucellosis in humans can serve as the first indicator of the disease in the animal population (Alton, 1990). Unlike in animals, identification of the causative agent to genus level is sufficient to initiate therapy, prevention, and control measures in the population. Human brucellosis may be suspected based on history of

exposure and clinical presentation. Serological screening and presumptive diagnosis are performed using several tests that are primarily targeted at detecting high or rising titres of agglutinating *Brucella* antibodies (IgM, IgG and IgA), the details of which have been extensively reviewed by Alton et al. (1988), Al Dahouk et al. (2003) and Araj (2010). The Rose Bengal test (RBT) or agglutination tests such as the standard agglutination test (SAT) are the primary screening tests worldwide (Al Dahouk et al., 2003; Lucero et al., 2005), while the IgG ELISA or Coombs IgG test or FPA are used as confirmatory test (WHO, 2005; Araj, 2010; Godfroid et al., 2010; Roushan et al., 2010). These tests, when ranked based on their accuracy in clinical settings, ELISA>RBT>SAT>CT (Al Dahouk, 2003). The SAT is reported to have a sensitivity and specificity of 87.4% and 100% respectively (Young, 1991; Gómez et al., 2008). Although Coomb's test is technically complicated and hence not a frequently used test, it is a good test for identifying chronic or relapsing *Brucella* infection (Ruiz-Mesa et al., 2005). Since serological tests are based on the identification of anti-LPS antibodies, cross-reactivity with other pathogens that have a similar immune-dominant epitope (O-polysaccharide) such as *Yersinia enterocolitica* O:9, *Salmonella urbana* group N, *Vibrio cholera, Francisella tularensis, Stenotrophomonas maltophilia* and *Escherichia coli* O157 may occur (Al Dahouk et al., 2003; Lucero et al., 2005; Ruiz-Mesa et al., 2005). Conventional serologic tests used for human brucellosis diagnosis are developed for smooth strains and hence, they cannot detect *B. canis* infection (Lucero et al., 2010). A commercial rapid agglutination test is the only assay used to detect *Brucella* infections in humans in Namibia.

As described for cattle, PCR methods are an effective tool for detecting *Brucella* infection, monitoring response to treatment and for identifying the infecting species. The detection of *Brucella* DNA in patients is difficult due to the low numbers of bacteria in infected tissue and the inhibitory effects of surrounding substances or tissue matrix (Al Dahouk et al., 2007b; Queipo-Ortuno et al., 2008). PCR can be performed on clinical specimens such as tissues, whole blood, cerebrospinal fluid, serum, pus, synovial or pleural fluid (Colmenero et al., 2005; Debeaumont et al., 2005; Queipo-Ortuño et al., 2005; 2006; 2009; Colmenero et al., 2010). At present, there is no validated PCR test for detecting *Brucella* infections in people in Namibia.

2.5. Prevention and control of brucellosis

2.5.1. Bovine brucellosis

Treatment of brucellosis in cattle is futile and not recommended, because of the long duration of treatment required; the inconsistent results achieved among the infected animals; the occurrence of antibiotic residues in meat and milk (Petzer et al., 1984) and the frequent disease relapses that occur due to the occasional release of bacteria from intracellular locations (Milward et al., 1984; Nicoletti et al., 1985; Nicoletti et al., 1989; Radwan et al., 1993). Therefore, strategies aimed at disease prevention, control or eradication are the attainable options for managing the disease.

The primary objective of brucellosis control programs is to reduce infection in the animal population to a level that minimizes the impact of the disease on the human population, animal health and production (EU, 2001; De Massis et al., 2005; Pappas et al., 2006). In other cases, brucellosis measures are targeted at eradicating the disease from a region or country. The most common methods employed to control brucellosis include test and slaughter, animal movement controls, quarantine, vaccination, biosecurity, eradication, and disease surveillance (McDermott and Arimi, 2002; Godfroid et al., 2004; Blasco and Molina-Flores, 2011; Spickler, 2018). The choice of which strategy or strategies to use depends on the prevailing epidemiological situation and availability of resources, among others (Nicoletti, 2010). To be effective, all strategies should be complemented by an effective animal identification and movement control system; a well-functioning disease surveillance and reporting system and a market related compensation system for culled animals (Crespo et al., 2012). Australia and New Zealand successfully eradicated brucellosis using a program based on individual animal identification, categorisation of cattle herds according to brucellosis status; controls on the movement of cattle between areas; regular monitoring of cattle herds; fair compensation for culled positive animals; optimized laboratory capacity; efficient record keeping and the training of relevant stakeholders (Shepherd et al., 1980; Tweddle and Livingstone, 1994). The FAO strategy for the control of brucellosis is based on a five-point action plan comprising a baseline sero-prevalence survey of animals based on statistical methods, development and implementation of a risk-based vaccination control

strategy, effective disease surveillance to ensure early warning against spread to new areas, monitoring results for progress and changes in infection/disease incidence, and reviewing and updating control strategies to reflect the results obtained (FAO, 2010).

Implementation of biosecurity measures prevents the spread of *Brucella* by excluding the entry or exit of pathogens from a livestock facility. These measures, which include hygiene practices, when fully implemented can prevent environmental contamination and exposure of susceptible animals to *Brucella* bacteria (Pérez-Sancho et al., 2015). Biosecurity measures include secure farm boundaries and restricted access to prevent contact with wild animals and livestock of unknown brucellosis status; strict quarantine and testing of new animals before they are introduced into the herd; purchasing of replacement heifers from brucellosis-free herds; sourcing semen for artificial insemination from *Brucella*-negative bulls as confirmed by results of regular tests (Spickler, 2018); rearing of own replacement animals (FAO, 2010); appropriate disposal of aborted fetuses, fetal membranes and manure; cleaning and disinfection of contaminated premises and equipment and of persons at farm entry points (FAO, 2006; Pérez-Sancho et al., 2015). Vermin and pests such as rodents and flies should be controlled by use of appropriate rodenticides, fly traps and baits as they can mechanically transmit brucellosis.

Control of animal movements within a region, country and across international boundaries prevents the spread of brucellosis between farms, regions, and countries with different brucellosis status. For movement controls to be effective, an effective individual cattle identification system that helps in quick identification of restricted animals should be implemented. Imported animals should be certified free of brucellosis before transportation from a country of origin (OIE, 2018; WHO, 2006) and before introduction onto the main herd. In many endemic areas, failure to control animal movements has been identified as a major obstacle to brucellosis control (McGiven, 2013). In resource poor countries, mass vaccinations, movement controls and public health education are the recommended approaches for the control of brucellosis (FAO, 2010).

Control of brucellosis requires sustained efforts that are often costly. As a result, the spectrum and extent of implementation of control measures varies between developed and developing countries, with the later constrained by a higher disease burden and resources including animal health infrastructure (Ragan, 2002; Kouba, 2003; Franco et al., 2007; Ibironke et al., 2008; Pavade et al., 2011; Howe et al., 2013; McDermott et al., 2013; Cárdenas et al., 2019). Resource limited countries cannot offer adequate incentives to farmers to participate in and achieve brucellosis-free certification of their herds (Moreno et al., 2002; Aznar et al., 2014) despite some of these countries having a sound legislative basis. Co-operation between government and the livestock industry is also key for participatory implementation of prevention, control, or eradication measures against brucellosis (More et al., 2015) as has been demonstrated in the Czech Republic (Kouba, 2003) and in the United States in domestic animals (Ragan, 2002).

2.5.1.1. The test and slaughter approach

In the test and slaughter approach, cattle herds are repeatedly tested by serology to identify positive cattle and herds. Positive reactors herds are placed on movement restriction for specified periods and serologically tested until they are certified as brucellosis-free. Two consecutive negative CFT results six months apart in all animals in a herd are accepted as adequate proof of eradication of the disease (Kolar, 1984; Alton, 1987). Individual reactor cattle are slaughtered or culled by regulatory officials following strict hygiene and biosecurity measures to decrease the incidence of infection, reduce the spread of infection within and between cattle herds (Pérez-Sancho et al., 2015; Li et al., 2017) and to provide public health protection. According to Nicoletti (1993), this approach is justified and most likely to succeed in regions or countries where the prevalence of bovine brucellosis is less than or equal to 2%. Where the prevalence of brucellosis is greater than 5%, a combination of the test-and-slaughter approach in adult cattle and vaccination of young replacement animals is recommended to eradicate the infection in the medium to long term (EU, 2001). For a test and slaughter approach to succeed, complimentary support in the form of an individual animal identification system; an efficient surveillance system; good laboratory diagnostic services; farmer compliance; strict animal movement control and quarantine systems should be provided (Alton, 1990; Nicoletti, 1993) as

a minimum. Furthermore, the test and slaughter approach should be supported by adequate financial resources to ensure fair compensation to farmers for culled reactor cattle (Moriyón et al., 2004; Ibironke et al., 2008; Ducrotoy et al., 2017; Cárdenas et al., 2017). The test and slaughter policy has been successfully used to eradicate brucellosis from cattle in several countries around the world (Kolar, 1984; Nicoletti, 1993).

2.5.1.2. Surveillance for brucellosis

Most countries have national guidelines for animal disease reporting that are based on local legislation. Around the world (and in Namibia) bovine brucellosis is a notifiable disease, which should be reported to the national authorities whenever it is suspected or confirmed. In Namibia, measures for the control of brucellosis are as prescribed in the Animal Health Act (Act 1 of 2011).

Continuous surveillance and monitoring enable early detection of changes in brucellosis incidence and prevalence (Thrusfield, 1995). This information is necessary for evaluating and improving disease prevention and control strategies by risk managers (WHO, 2006; FAO, 2010). Surveillance systems should be implemented systematically and as a minimum should integrate serological testing and clinical surveillance by veterinary personnel (FAO, 2006) at herd, region, and national level. In addition to farms, routine cattle gathering places such as abattoirs, feedlots and auctions are ideal places for brucellosis surveillance. A system should be instituted to ensure that all cases of abortions are reported and investigated and that samples are submitted to the laboratory for confirmation of diagnosis. Appropriate action should be taken against any infected cattle identified. Around the world, as in Namibia, surveillance systems for brucellosis are implemented well on dairy cattle than on beef cattle farms (de Alencar et al., 2016).

Surveillance for brucellosis in Namibia is based on the inspection of cattle at animal gatherings such as farms, auctions and at abattoirs by animal health technicians and veterinarians; investigation of abortion cases reported by farmers including submission of samples for confirmation at the laboratory; and serological testing (mandatory and voluntary testing). On farms, veterinary officials examine farm

records and query farmers for evidence and history of abortions, and inspect live animals. On dairy farms, annual serological testing of cattle is compulsory, while on beef farms, serological testing is voluntary. *Brucella*-positive herds are placed under movement restrictions (no movement of cattle into or out of the farm), and all in-contact animals are tested and retested until all animals in the herd test negative. Seropositive cattle are culled or slaughtered at a designated abattoir under the supervision of a veterinary official, taking into consideration the measures that are necessary to prevent infection of slaughter men.

2.5.1.3. Control of brucellosis by vaccination

Vaccination is a key, effective and indispensable strategy for the prevention and control of *Brucella* infections in cattle worldwide (Olsen and Stoffregen, 2005). To be effective, vaccination should be implemented in conjunction with other measures such as biosecurity, surveillance measures and test-and-slaughter policies (Olsen and Stoffregen, 2005; Nicoletti, 2010). Vaccination increases herd immunity, eliminates clinical signs of brucellosis including abortions, reduces environmental contamination with *Brucella* bacteria (Nicoletti, 1993) and prevents the spread of infections within and between cattle herds. Vaccination of cattle herds is considered as the most appropriate and cost-effective measure in the early stages of brucellosis control in endemic regions when the prevalence of the disease is still high (Zinsstag et al., 2007; McDermott et al., 2013). A combination of vaccination of heifers, testing and slaughter of positive reactors is a good basis for controlling bovine brucellosis in endemic areas (Nicoletti, 2010). The success of a vaccination program for the control of brucellosis depends on vaccine quality, efficacy, and vaccination coverage in the target population (Nicoletti, 1990). All vaccines currently in use against *Brucella* infection are live attenuated strains because killed vaccines have been proven to be ineffective at stimulating a protective immune response (Nicoletti, 1990). *Brucella abortus* S19 and *B. abortus* strain RB51 are the two vaccines that are widely used in bovine brucellosis control programs (Frolich et al., 2002; Martins et al., 2009). Each of the two vaccines has unique advantages and disadvantages, but S19 is reported to be superior to RB51 (FAO, 2006). Although both vaccines elicit the production of protective antibodies, they cannot prevent infections by field strains of *Brucella* (Olsen and Stoffregen, 2005).

Brucella abortus S19 is highly effective at preventing abortions and subsequent transmission of the disease. Antibodies induced by S19 vaccination and natural infection are directed at the S-LPS (O side chain) and thus indistinguishable by most serological tests (Olsen and Stoffregen, 2005). *Brucella abortus* S19 once off vaccination is limited to heifers of 4-8 months of age (3-8 months in Namibia) as a subcutaneous dose of 5-8 x 10^{10} viable brucellae (OIE, 2018). The major limitation of S19 vaccine relates to the occasional persistence of circulating antibodies post-vaccination, which may confuse the interpretation of diagnostic serological tests (Schurig et al., 2002; FAO, 2006; Godfroid et al., 2011). In general, following calfhood vaccination, S19 does not persist in the uterus of mature heifers or cause abortions, but may cause abortions when administered to pregnant cows (Godfroid et al., 2011). *Brucella abortus* S19 induces longer term immunity than RB51 in female animals (Singh et al., 2012; Dorneles et al., 2015; Miranda et al., 2015). Full dose S19 vaccination has been reported to induce antibodies that last the production lifetime of cattle- up to 4.5 years (Simpson et al., 2018).

RB51 vaccine is a rough mutant prepared from a virulent smooth *B. abortus* biovar 1 strain 2308 which lacks an O-side chain (Schurig et al., 1991). Therefore, RB51 does not interfere with serological tests (USDA, 2003; Sanz et al., 2010; Spickler, 2018) because it elicits the production of antibodies to the LPS lipid A-core (Ducrotoy et al., 2016). As a result, vaccinated and naturally infected cattle can be differentiated on most serological tests (Schurig et al., 2002; Vemulapalli et al., 2002). Unlike S19, RB51 allows for the vaccination of cows and for booster vaccinations to be administered without interfering with serological tests (Oberem et al., 2006) and does not induce placentitis and abortions in pregnant animals (Schurig et al., 2002). However, rare reports of RB51 vaccine causing infection in cows (Arellano Reynoso et al., 2013) due to residual virulence (Olsen and Tatum, 2010; Van Metre, 1999) have been documented raising public health concern because the RB51 strain is resistant to rifampicin (Anonymous, 1998).

Other vaccines that have been tested or used widely for vaccinating cattle against brucellosis are *B. abortus* 45/20, *B. abortus* SR82 (Olsen and Stoffregen, 2005), and

an S2 vaccine that is used widely in China (Jiang et al., 2018). Heat-killed *B. abortus* biovar 1 strain 45/20 has been used in some European countries to overcome the drawbacks of S19 without success. The live attenuated SR82 vaccine prepared from *B. abortus* biovar 6 has a protection level similar to S19, and is effective under field conditions (Ivanov et al., 2011). It is widely used against bovine brucellosis in Azerbaijan, the Russian Federation and Tajikistan (Ivanov et al., 2011).

In Namibia, compulsory vaccination of all heifers 3-8 months old with S19 vaccine or of both heifers and cows with RB51 vaccine is the basis of brucellosis control in the country (Animal Health Act, 2011).

2.5.1.4. Decontamination of infected material

All *Brucella* infected material and products of abortion should be collected with care and disposed of safely by incineration, burning or deep burial with lime away from water courses. Dung, dust, soil, and farm implements on dairy farms that are contaminated with *Brucella* organisms may be disinfected using iodophor, formaldehyde, 70% ethanol, phenolic or hypochlorite disinfectants (Spickler, 2018). Contaminated dung can harbour *Brucella* organisms for a long time and should therefore be cleared and stored in a secluded area where the natural decomposition process will kill the bacteria, a process which may take up to 12 months. The addition of xylene to liquid dung has been reported to hasten the destruction of *Brucella*.

2.5.2. Prevention and control of human brucellosis

Brucellosis is both a preventable and curable infectious disease of humans, whose control is based on early diagnosis and aggressive antibiotic treatment. Prevention and control of brucellosis involves two main strategies, that is, prevention of exposure to infected animals and fomites, and occupational exposure, and maintaining food safety in animal products. Due to the broad effects of the brucellosis on the environment, human and animal populations, a One Health approach towards the control of the disease involving relevant professions such as the veterinary, medical, public health, cultural, economic and social experts is necessary to effectively combat the infection (Bamaiyi, 2016; Franc et al., 2018).

2.5.2.1. Prevention of exposure

Control of brucellosis in livestock results in the control of the disease in humans (Vemulapalli et al., 2004; Seleem et al., 2010). In occupationally exposed professions, infection can be prevented by putting on adequate protective gear such as overalls or coats, rubber or plastic aprons, rubber gloves, eye protection (Spickler, 2018) especially when slaughtering or handling high risk or known *Brucella*-positive cattle (WHO, 2006). All wounds and cuts should be covered with impervious wound dressing (FAO, 2006; Spickler, 2018) or alternatively, affected personnel should be excluded from handling animals and animal products until they are healed. The administration of cattle vaccines should be done with care to prevent accidental inoculation or contamination of mucous membranes or abraded skin (Spickler, 2018). Contaminated laboratory equipment should be routinely cleaned and sterilised to reduce the risk of infection among clinical laboratory workers (Roy et al., 2011).

2.5.2.2. Food safety

Humans are infected by *Brucella* mainly through inappropriately prepared and/or preserved food of animal origin. Pasteurization of dairy products including raw milk kills brucellae and is thus considered as one of the main public health measures against zoonotic infections of brucellosis (Hull and Schumaker, 2018). However, pasteurisation or boiling of milk before consumption is not routinely practised in resource-limited communities because of long-standing cultural practices and a general lack of understanding of the dangers of consuming raw milk (Welburn et al., 2015). In low- and middle-income countries, the lack of effective controls and enforcement of standards on products sold at informal markets increases the risk of transmission of diseases, including brucellosis to humans through food (Grace, 2015). In addition, informal traders lack awareness and knowledge of the risk posed by raw milk to human health and cannot afford the cost of the equipment required for pasteurization (Cosivi et al., 1998; Welburn et al., 2015). The boiling or heating of raw milk to at least 80^{o}C for several minutes is a feasible strategy that can be promoted to prevent human brucellosis especially in resource limited areas where the disease is endemic (Godfroid et al., 2004; Spickler, 2018). Hard cheeses that

undergo fermentation processes during production are less of a risk to humans. The World Health Organization (WHO) recommends that soft cheeses produced from unpasteurized milk be stored for more than 6 months before they are consumed. Meat, blood, and internal organs from animals of unknown brucellosis status do pose a risk of infection and should therefore always be handled with care and thoroughly cooked prior to consumption (Spickler, 2018). Rennet used in the cheese making process should be sourced from *Brucella*-free animals to prevent human infections. Butter, sour milk, sour cream, and yoghurt are dairy products that undergo acidification processes which reduce the *Brucella* content. For effective killing of *Brucella*, the pH of the product should be less than 3.5.

2.5.2.3. Treatment of human brucellosis

Treatment of brucellosis in humans is based on antibiotic combinations as has been described by the World Health Organisation (2005). Several conventional antimicrobial agents such as tetracycline, trimethoprim-sulfamethoxazole, aminoglycosides, rifampicin, quinolones, doxycycline, and streptomycin (Saltoglu et al., 2002) are commonly used. A combination of 100 mg of doxycycline twice a day and rifampin (15 mg/kg/day) for 45 days (six weeks) is effective (Ersoy et al., 2005; Solis Garcia del Pozo and Solera, 2012; Hartady et al., 2014; Kaya et al., 2018; Meng et al., 2018; Yang et al., 2018) and recommended by the World Health Organisation (Corbel, 2006) for the treatment of human brucellosis. Alternatively, a combination of 100 mg of doxycycline orally twice a day for 45 days and 1g daily of streptomycin intramuscularly for the first 15-21 days of treatment has also been reported to be effective (Corbel, 2006; Seleem et al., 2010) and to have a lower relapse rate than the doxycycline-rifampin combination (Ariza et al., 1985; Skalsky et al., 2008). Other alternative combination therapies include doxycycline-rifampin-gentamycin (Skalsky et al., 2008), trimethoprim-sulphamethoxazole (Karabay et al., 2004; Solera, 2010), doxycycline-fluoroquinolone and rifampin-ciprofloxacin (Solis Garcia del Pozo and Solera, 2012). Monotherapy with co-trimoxazole, rifampicin or gentamycin has also been successfully used to treat brucellosis in pregnant women (Karabay et al., 2004; WHO, 2005; FAO, 2006). In cases of accidental inoculation with live *Brucella* vaccines, a six-week course of doxycycline is recommended in

addition to wound management and tetanus toxoid injection (FAO, 2006). Localized forms of the disease such as endocarditis may require surgery (FAO/WHO, 2006).

2.5.2.4. Public health education

Implementation of laws, regulations and veterinary policy measures may not bring the desired results, if the population at large, is not aware of the disease (FAO, 2003). Targeted public health education to improve awareness of this zoonotic disease is necessary as a preventive measure especially in endemic resource poor regions (Marcotty et al., 2009; FAO, 2010; Asiimwe et al., 2015). Education of people directly involved or at risk in various spheres of society including schools, the animal and food industry, such as farmers, butchers and abattoir workers empowers them with the knowledge to take up responsibility in preventing brucellosis within their environment. Medical personnel such as physicians and nurses who are at the forefront of human disease surveillance often lack knowledge of zoonotic diseases such as brucellosis to make a diagnosis (Hesterberg et al., 2008). This can be addressed through continuous professional training and adequate inter-sectoral collaboration between the relevant governmental and intergovernmental organizations (FAO, 2010). Studies in Asia found that human populations that are aware of the mode of transmission or the need for pasteurization of milk and other dairy products have significantly reduced risk of *Brucella* infection (FAO, 2010). The latest knowledge on brucellosis needs to be transferred to all stakeholders, especially those located in rural populations, to curb the spread of brucellosis (Donev, 2010; Franc et al., 2018).

2.5.2.5. Vaccination against human brucellosis

There are currently no safe, effective, reliable licensed vaccines for the immunization of humans against brucellosis (Godfroid et al, 2011; Surendran et al., 2011).

2.6. References

ABERNETHY, D.A., MENZIES, F.D., MCCULLOUGH, S.J., MCDOWELL, S.W., BURNS, K.E., WATT, R., GORDON, A.W., GREINER, M. & PFEIFFER, D.U. 2012. Field trial of six serological tests for bovine brucellosis. *Veterinary Journal*, 191(3), 364-370. DOI:10.1016/j.tvjl.2011.03.008

AGASTHYA, A.S., ISLOOR, S. & KRISHNAMSETTY, P. 2012. Seroprevalence study of human brucellosis by conventional tests and indigenous Indirect Enzyme-Linked Immunosorbent Assay. *Scientific World Journal*, 2012, 104239. DOI:10.1100/2012/104239.

AL DAHOUK, S., TOMASO, H., NÖCKLER, K., NEUBAUER, H. & FRANGOULIDIS, D. 2003. Laboratory based diagnosis of brucellosis – a review of the literature. Part II: serological tests for brucellosis. *Clinical Laboratory*, 49(11–12), 577–589.

AL DAHOUK, S., LE FLÈCHE, P., NÖCKLER, K., JACQUES, I., GRAYON, M., SCHOLZ, H.C., TOMASO, H., VERGNAUD, G. & NEUBAUER, H. 2007a. Evaluation of *Brucella* MLVA typing for human brucellosis. *Journal of Microbiological Methods*, 69(1), 137–145. DOI: 10.1016/j.mimet.2006.12.015.

AL DAHOUK, S., NOCKLER, K., SCHOLZ, C.H., PFEFFER, M., NEUBAUER, H. & TOMASO, H. 2007b. Evaluation of genus-specific and species-specific real-time PCR assays for the identification of *Brucella* spp. *Clinical Chemistry and Laboratory Medicine*, 45, 1464–1470. DOI: 10.1515/CCLM.2007.305.

AL DAHOUK, S., SCHOLZ, H.C., TOMASO, H., BAHN, P., GÖLLNER, C., KARGES, W., APPEL, B., HENSEL, A., NEUBAUER, H. & NÖCKLER, K. 2010. Differential phenotyping of *Brucella* species using a newly developed semi-automated metabolic system. *BMC Microbiology*, 10, 269. DOI: 10.1186/1471-2180-10-269.

AL DAHOUK, S. & NÖCKLER, K. 2011. Implications of laboratory diagnosis on brucellosis therapy. *Expert Review of Anti-infective Therapy*, 9, 833–845. DOI: 10.1586/eri.11.55.

AL DAHOUK, S., KÖHLER, S., OCCHIALINI, A., JIMÉNEZ DE BAGUËŚ, M.P., HAMMER, J.A., EISENBERG, T., VERGNAUD, G., CLOECKAERT, A., ZYGMUNT, M.S., WHATMORE, A.M., MELZER, F., DREES, K.P., FOSTER, J.T., WATTAM, A.R. & SCHOLZ, H.C. 2017. *Brucella* spp. of amphibians

comprise genomically diverse motile strains competent for replication in macrophages and survival in mammalian hosts. *Scientific Reports*, 7, 44420. DOI: 10.1038/srep44420.

AL-MAJALI, A.M., AL-QUDAH, K.M., AL-TARAZI, Y.H. & AL-RAWASHDEH, O.F. 2008. Risk factors associated with camel brucellosis in Jordan. *Tropical Animal Health and Production*, 40, 193-200. DOI: 10.1007/s11250-007-9080-7.

ALP, E. & DOGANAY, M. 2008. Current therapeutic strategy in spinal brucellosis. *International Journal of Infectious Diseases*, 12, 573–577. DOI: 10.1016/j.ijid.2008.03.014.

ALTON, G.G. 1987. Control of *Brucella melitensis* infection in sheep and goats - a review. *Tropical Animal Health and Production*, 19, 65-74. DOI: 10.1016/j.cvfa.2010.10.003.

ALTON, G.G., JONES, L.M., ANGUS, R.D. & VERGER, J.M. 1988. Techniques for the Brucellosis Laboratory. Institut National de la Recherche Agronomique, Paris, France. pp. 81-134.

ALTON, G.G. 1990. In: DUNCAN, J.R. & NIELSEN, K. eds. Animal Brucellosis. Boston: 383-409.

ALTON, G.G. & FORSYTH, J.R.L. 1996. *Brucella* (Chapter 28). In: Baron, S (ed), Galveston: University of Texas Medical Branch.

AMIN, A.S., HAMDY, M.E. & IBRAHIM, A.K. 2001. Detection of *Brucella melitensis* in semen using the polymerase chain reaction assay. *Veterinary Microbiology*, 83(1), 37–44. DOI: 10.1016/s0378-1135(01)00401-1.

ANKA, M.S., HASSAN, L., KHAIRANI-BEJO, S., ZAINAL, M.A., MOHAMAD, R.B., SALLEH, A. & ADZHAR, A. 2014. A case-control study of risk factors for bovine brucellosis seropositivity in Peninsular Malaysia. PLoS ONE, 9(9), e108673. DOI: 10.1371/journal.pone.0108673.

ANONYMOUS. 1998. Human exposure to *Brucella abortus* strain RB51--Kansas, 1997. MMWR. *Morbidity and Mortality Weekly Report*, 47, 172-175.

ARAJ, G.F. 1999. Human brucellosis: a classical infectious disease with persistent diagnostic challenges. *Clinical Laboratory Science*, 12, 207-212.

ARAJ, G.F. 2010. Update on laboratory diagnosis of human brucellosis. *International*

Journal of Antimicrobial Agents, 36, 12–17. DOI: 10.1016/j.ijantimicag.2010.06.014.

ARELLANO-REYNOSO, B., SUÁREZ-GÜEMES, F., ESTRADA, F.M., MICHEL-GÓMEZFLORES, F., HERNÁNDEZ-CASTRO, R., ACOSTA, R.B. & DIAS-APARICIO, E. 2013. Isolation of a field strain of *Brucella abortus* from RB51-vaccinated- and brucellosis-seronegative bovine yearlings that calved normally. *Tropical Animal Health and Production*, 45, 695–697. DOI: 10.1007/s11250-012-0252-8.

ARIF, S., THOMSON, C.P., HERNANDEZ-JOVER, M., MCGILL, M.D., WARRIACH, M.H. & HELLER, J. 2017. Knowledge, attitudes, and practices (KAP) relating to brucellosis in smallholder dairy farmers in two provinces in Pakistan. *PLoS ONE*, 12, e0173365. DOI: 10.1371/journal.pone.0173365.

ARIZA, J., GUDIOL, F., PALLARÉS, R., RUFÍ, G. & FERNÁNDEZ-VILADRICH, P. 1985. Comparative trial of rifampin-doxycycline versus tetracycline-streptomycin in the therapy of human brucellosis. *Antimicrobial Agents and Chemotherapy,* 28, 548-551. DOI: 10.1128/aac.28.4.548.

ASIIMWE, B.B., KANSIIME, C. & RWEGO, I.B. 2015. Risk factors for human brucellosis in agro-pastoralist communities of south western Uganda: a case-control study. *BMC Research Notes*, 8, 405. DOI: 10.1186/s13104-015-1361-z.

AWAH-NDUKUM, J., MOUICHE, M.M.M., BAYANG, H.N., NGUNGWA, V., ASSANA, E., FEUSSOM, K.J.M., MANCHANG, T.K. & ZOLI, P.A. 2018. *Veterinary Medicine International*. DOI: 10.1155/2018/3468596.

AZNAR, M.N., SAMARTINO, L.E., HUMBLET, M.F. & SAEGERMAN, C. 2014. Bovine brucellosis in Argentina and bordering countries: update. *Transboundary and Emerging Diseases*, 61(2), 121 – 133. DOI: 10.1111/tbed.12018.

BAMAIYI, P.H. 2016. Prevalence and risk factors of brucellosis in man and domestic animals: a review. *International Journal of One Health*, 2, 29–34. DOI: 10.14202/IJOH.2016.29-34.

BEKELE, M., MOHAMMED, H., TEFERA, M. & TOLOSA, T. 2011. Small ruminant

brucellosis and community perception in Jijiga district, Somali Regional State, eastern Ethiopia. *Tropical Animal Health and Production*, 43, 893-898. DOI: 10.1007/s11250-011-9781-9.

BERCOVICH, Z., HAAGSMA, J. & TER LAAK, EA. 1990. Use of delayed-type hypersensitivity test to diagnose brucellosis in calves born to infected dams. *Veterinary Quarterly*, 12(4), 231-237. DOI: 10.1080/01652176.1990.9694270.

BERCOVICH, Z. & TAAIJKE, R. 1990. Enzyme immunoassay using mouse monoclonal anti-bovine antibodies for the detection of *Brucella abortus* antibodies in cow milk. *Journal of Veterinary Medicine*, Series B, 37(10), 753-759. DOI: 10.1111/j.1439-0450.1990.tb01124.x.

BERCOVICH, Z. 1998. Maintenance of *Brucella abortus* free herds: a review with emphasis on epidemiology and the problems of diagnosing brucellosis in areas of low prevalence. *Veterinary Quarterly*, 20, 81-88. DOI: 10.1080/01652176.1998.9694845.

BERGER, S. 2016. Brucellosis: Global Status. Los Angeles, CA: GIDEON Informatics, Inc.

BERGEY, D.H. & HOLT, J.G. 1994. Bergey's manual of determinative bacteriology. 9. Williams & Wilkins; Baltimore, MD, USA: 1994.

BISHOP, G.C., BOSMAN, P.P. & HERR, S. 1994. Bovine brucellosis. In J.A.W. COETZER, G.R. THOMSON & R.C. TUSTIN (eds.), Infectious Diseases of livestock with special reference to Southern Africa, pp. 1053-1066, Oxford University Press, Cape Town.

BLASCO, J.M., GARIN-BASTUJI, B., MARÍN, C.M., GERBIER, G., FANLO, J., JIMÉNEZ DE BAGÜES, M.P. & CAU, C. 1994. Efficacy of different Rose Bengal and complement fixation antigens for the diagnosis of *Brucella melitensis* infection in sheep and goats. *Veterinary Record*, 134, 415–420. DOI: 10.1136/vr.134.16.415.

BLASCO, J.M. & MOLINA-FLORES, B. 2011. Control and eradication of *Brucella melitensis* infection in sheep and goats. *Veterinary Clinics of North America: Food Animal Practice*, 27(1), 95-104. DOI: 10.1016/j.cvfa.2010.10.003.

BOGDANOVICH, T., SKURNIK, M., LÜBECK, P.S., AHRENS, P. & HOORFAR, J. 2004. Validated 5' Nuclease PCR assay for rapid identification of the genus *Brucella*. *Journal of Clinical Microbiology*, 42, 2261–2263.

DOI: 10.1128/jcm.42.5.2261-2263.2004

BOSCHIROLI, M.L., FOULONGNE, V. & O'CALLAGHAN, D. 2001. Brucellosis: A worldwide zoonosis. *Current Opinion in Microbiology*, 4, 58–64. DOI: 10.1016/s1369-5274(00)00165-x.

BOUKARY, A.R., THYS, E., ABATIH, E., GAMATIÉ, D., ANGO, I., YENIKOYE, A. & SAEGERMAN, C. 2011. Bovine tuberculosis prevalence survey on cattle in the rural livestock system of Torodi (Niger). *PLoS One*, 6(9), e24629.

BOUKARY, A.R., SAEGERMAN, C., ABATIH, E., FRETIN, D., ALAMBÉDJI BADA, R., DE DEKEN, R., HAROUNA, H.A., YENIKOYE, A. & THY, E. 2013. Seroprevalence and potential risk factors for *Brucella* spp. infection in traditional cattle, sheep and goats reared in urban, peri-urban and rural areas of Niger. *PLoS ONE*, 8(12), e83175. DOI: 10.1371/journal.pone.0083175.

BOUZA, E., SANCHEZ-CARRILLO, C., HERNANGOMEZ, S. & GONZALEZ, J.M. 2005. Laboratory-acquired brucellosis: A Spanish national survey. *Journal of Hospital Infection*, 61, 80–83. DOI: 10.1016/j.jhin.2005.02.018.

BRICKER, B.J. & HALLING, S.M. 1994. Differentiation of *Brucella abortus* bv 1, 2 and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella suis* bv 1 by PCR. *Journal of Clinical Microbiology*, 32, 2660–6. DOI:10.1128/JCM.32.11.2660-2666.1994.

BRICKER, B.J. & HALLING, S.M. 1995. Enhancement of the *Brucella* AMOS PCR assay for differentiation of *Brucella abortus* vaccine strains S19 and RB51. *Journal of clinical microbiology*, *33*(6), 1640–1642. DOI:10.1128/JCM.33.6.1640-1642.1995.

BRICKER, B.J. 2002. PCR as a diagnostic tool for brucellosis. *Veterinary Microbiology*, 90, 435–446. DOI: 10.1016/s0378-1135(02)00228-6.

BUZGAN, T., KARAHOCAGIL, M.K., IRMAK, H., BARAN, A.I., KARSEN, H., EVIRGEN, O. & AKDENIZ, H. 2010. Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. *International Journal of Infectious Disease*, 14(6), e469–78. DOI: 10.1016/j.ijid.2009.06.031.

CAMINITI, A., PELONE, F., BATTISTI, S., GAMBERALE, F., COLAFRANCESCO, R., SALA, M., LA TORRE, G., DELLA MARTA, U. & SCARAMOZZINO, P. 2017. Tuberculosis, brucellosis and leucosis in cattle: A cost description of

eradication programmes in the region of Lazio, Italy. *Transboundary Emerging Diseases*, 64, 1493–1504. DOI: 10.1111/tbed.12540.

CÁRDENAS, L., MELO, O. & CASAL, J. 2017. Evolution of bovine brucellosis in Colombia over a 7-year period (2006–2012). *Tropical Animal Health and Production*, 50(1), 19– 27. DOI: 10.1007/s11250-017-1395-4.

CÁRDENAS, L., PEÑA, M., MELO, O. & CASAL, J. 2019. Risk factors for new bovine brucellosis infections in Colombian herds. *BMC Veterinary Research,* 15**,** 81. DOI: 10.1186/s12917-019-1825-9.

CARRERA, I.A., RODRÍGUEZ, M.J.L., SAPIÑA, A.M., LAFUENTE, A.L. & SACRISTÁN, A.R.B. 2006. Probable transmission of brucellosis by breast milk. *Journal of Tropical Paediatrics*, 52(5), 380-381. DOI: 10.1093/tropej/fml029.

CARVALHO NETA, A.V., MOL, J.P., XAVIER, M.N., PAIXÃO, T.A., LAGE, A.P. & SANTOS, R.L. 2010. Pathogenesis of bovine brucellosis. *Veterinary Journal*, 184(2), 146–155. DOI: 10.1016/j.tvjl.2009.04.010.

CASTANO, M.J. & SOLERA, J. 2009. Chronic brucellosis and persistence of *Brucella melitensis* DNA. *Journal of Clinical Microbiology*, 47, 2084–2089. DOI: 10.1128/JCM.02159-08.

CATLIN, J.E. & SHEEHAN, E.J. 1986. Transmission of bovine brucellosis from dam to offspring. *Journal of the American Veterinary Medical Association*, 188, 867–869.

CELEBI, G., KÜLAH, C., KILIÇ, S. & ÜSTÜNDAG˘, G. 2007. Asymptomatic *Brucella* bacteraemia and isolation of *Brucella melitensis* biovar 3 from human breast milk. *Scandinavian Journal of Infectious Diseases*, 39, 205–208. DOI: 10.1080/00365540600978898.

COELHO, A.M., DÍEZ, J.G. & COELHO, A.C. 2013. Brucelosis en pequeños rumiantes: efecto de la aplicación de un programa especial de vacunación en masa con REV-1. REDVET. *Revista Electrónica de Veterinaria*, 14, 1-16.

COELHO, A.C., DÍEZ, J.G. & COELHO, A.M. 2015. Risk Factors for *Brucella* spp. in domestic and wild animals. In Updates on Brucellosis, Baddour, M.M. (eds). IntechOpen. DOI: 10.5772/61325. https://www.intechopen.com/books/updates-on-brucellosis/risk-factors-for-brucella-spp-in-domestic-and-wild-animals.

COLMENERO, J.D., QUEIPO-ORTUÑO, M.I., REGUERA, J.M., BAEZA, G., SALAZAR, J.A. & MORATA, P. 2005. Real time polymerase chain reaction: a new powerful tool for the diagnosis of neurobrucellosis. *Journal of Neurology, Neurosurgery, and Psychiatry*, 76(7), 1025–1027. DOI: 10.1136/jnnp.2004.049411.

COLMENERO, J.D., MORATA, P., RUIZ-MESA, J.D., BAUTISTA, D., BERMÚDEZ, P., BRAVO, M.J. & QUEIPO-ORTUÑO, M.I. 2010. Multiplex real-time polymerase chain reaction: a practical approach for rapid diagnosis of tuberculous and brucellar vertebral osteomyelitis. *Spine*, 35(24), 1392–1396. DOI: 10.1097/BRS.0b013e3181e8eeaf.

CORBEL, M.J. & BRINLEY-MORGAN, W.J. 1984, Genus *Brucella* (Meyer and Shaw 1920), in N.R. KRIEG and J.G. HOLT (eds.), Bergey's Manual of Systematic Bacteriology, pp. 377-388, Williams and Wilkins, Baltimore, London.

CORBEL, M.J. 1997. Brucellosis. An overview. *Emerging Infectious Diseases*, 3(2), 213–221. DOI: 10.3201/eid0302.970219.

CORBEL, M.J. & BANAI, M. 2005. Bergey's Manual of systematics of Archaea and bacteria. John Wiley and Sons Inc.

CORBEL, M.J. 2006. Treatment of human brucellosis, in CORBEL, M.J., ELBERG, S.S. & COSIVI, O. (eds). Brucellosis in Humans and Animals (Chapter 5), pp. 36-41. World Health Organization, Geneva, Switzerland.

COSIVI, O., GRANGE, J., DABORN, C., RAVIGLIONE, M.C., FUJIKURA, T., COUSINS, D., ROBINSON, R.A., HUCHZERMEYER, H.F., DE KANTOR, I. & MESLIN, F.X. 1998. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerging Infectious Diseases*, 4(1), 59. DOI: 10.3201/eid0401.980108.

CRAWFORD, R.P., HUBER, J.D. & ADAMS, B.S. 1990. Epidemiology and surveillance. *Animal brucellosis*, 91, 131-151.

CRESPO, L.F., SAEZ, L.J.L., REVIRIEGO, G.F.J., RODRIGUEZ, F.E.F. & DURAN, F.M. 2012. Complementary tools for the control and eradication of caprine and ovine brucellosis in the European Union. *Revue Scientifique et Technique (International Office of Epizootics*), 31(3), 985-996. DOI: 10.20506/rst.31.3.2174.

CFSPH. 2007. Brucellosis.

http://www.cfsph.iastate.edu/Factsheets/pdfs/brucellaovis.pdf. Accessed November 10, 2018.

CURRÒ, V., MARINEO, S., VICARI, D., GALUPPO, L., GALLUZZO, P., NIFOSÌ, D., PUGLIESE, M., MIGLIAZZO, A., TORINA, A. & CARACAPPA, S. 2012. The isolation of *Brucella* spp. from sheep and goat farms in Sicily. *Small Ruminant Research*, 106, S2–S5. DOI: 10.1016/j.smallrumres.2012.04.025.

DAGLI, O., DOKUR, M., GUZELDAG, G. & OZMEN, Y. 2011. Acute renal failure due to *Brucella melitensis*. *Journal of Infection in Developing Countries*, 5(12), 893–5. DOI: 10.3855/jidc.1442.

DE ALENCAR, M.A.L.A., FERREIRA, F., NETO, J.S., R.A., AMAKU, M., GRISI-FILHO, J.H., TELLES, E.O. & GONÇALVES, V.S.P. 2016. Large-scale study of herd-level risk factors for bovine brucellosis in Brazil. *Acta Tropica*, 164, 226–232. DOI: 10.1016/j.actatropica.2016.09.016.

DE FIGUEIREDO, P., FICHT, T.A., RICE-FICHT, A., ROSSETTI, C.A. & ADAMS, L.G. 2015. Pathogenesis and immunobiology of brucellosis: Review of *Brucella*-host interactions. *American Journal of Pathology*, 185, 1505-1517. DOI: 10.1016/j.ajpath.2015.03.003.

DE MASSIS, F., GIOVANNINI, A., DI EMIDIO, B., RONCHI, G.F., TITTARELLI, M., DI VENTURA, M., NANNINI, D. & CAPORALE, V. 2005. Use of the complement fixation and brucellin skin tests to identify cattle vaccinated with *Brucella abortus* strain RB51. *Veterinaria Italia*, 41 (4), 291–299.

DE MIGUEL, M.J., MARÍN, C.M., MUÑOZ, P.M., DIESTE, L., GRILLÓ, M.J. & BLASCO J.M. 2011. Development of a selective culture medium for primary isolation of the main *Brucella* species. *Journal of Clinical Microbiology*, 49, 1458–1463. DOI: 10.1128/JCM.02301-10.

DE, B. K., STAUFFER, L., KOYLASS, SHARP, S.E., GEE, J.E., HELSEL, L.O., STEIGERWALT, A.G., VEGA, R., CLARK, T.A., DANESHVAR, M.I., WILKINS, P.P. & WHATMORE, A.M. 2008. Novel Brucella strain (BO1) associated with a prosthetic breast implant infection. *Journal of Clinical Microbiology*, 46, 43–49. DOI: 10.1128/JCM.01494-07.

DEAN, A.S., CRUMP, L., GRETER, H., SCHELLING, E. & ZINSSTAG, J. 2012a. Global burden of human brucellosis: A systematic review of disease frequency. *PLOS Neglected Tropical Diseases*, 6(10), e1865.

DOI: 10.1371/journal.pntd.0001865.

DEAN, A.S., CRUMP, L., GRETER, H., HATTENDORF, J., SCHELLING, E. & ZINSSTAG, J. 2012b. Clinical manifestations of human brucellosis: a systematic review and meta-analysis. *PLoS Neglected Tropical Diseases,* 6(12), e1929. DOI: 10.1371/journal.pntd.0001929.

DEBEAUMONT, C., FALCONNET, P.A. & MAURIN, M. 2005. Real-time PCR for detection of *Brucella* spp. DNA in human serum samples. *European Journal of Clinical Microbiology and Infectious Disease*, 24(12), 842–845. DOI: 10.1007/s10096-005-0064-0.

DELVECCHIO, V.G., KAPATRAL, V., REDKAR, R.J., PATRA, G., MUJER, C., LOS, T., IVANOVA, N., ANDERSON, I., BHATTACHARYYA, A., LYKIDIS, A., REZNIK, G., JABLONSKI, L., LARSEN, N., D'SOUZA, M., BERNAL, A., MAZUR, M., GOLTSMAN, E., SELKOV, E., ELZER, P.H., HAGIUS, S., O'CALLAGHAN, D., LETESSON, J.J., HASELKORN, R., KYRPIDES, N. & OVERBEEK, R. 2002. The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. *Proceedings of the National Academy of Sciences USA*, 99, 443–448. https://www.jstor.org/stable/3057556.

DENTINGER, C.M., JACOB, K., LEE, L.V., MENDEZ, H.A., CHOTIKANATIS, K., MCDONOUGH, P.L., CHICO, D.M., DE, B.K., TILLER, R.V., TRAXLER, R.M., CAMPAGNOLO, E.R., SCHMITT, D., GUERRA, M.A. & SLAVINSKI, S.A. 2014. Human *Brucella canis* infection and subsequent laboratory exposures associated with a puppy, New York City, 2012. *Zoonoses and Public Health*, 62(5), 407-414. DOI: 10.1111/zph.12163.

DÍAZ-APARICIO, E., MARÍN, C., ALONSO-URMENETA, B., ARAGÓN, V., PÉREZ-ORTIZ, S., PARDO, M. & MORIYÓN, I. 1994. Evaluation of serological tests for diagnosis of *Brucella melitensis* infection of goats. *Journal of Clinical Microbiology*, 32, 1159-1165. DOI: 10.1128/JCM.32.5.1159-1165.1994.

DI FEBO, T., LUCIANI, M., PORTANTI, O., BONFINI, B., LELLI, R. & TITTARELLI, M. 2012. Development and evaluation of diagnostic tests for the serological diagnosis of brucellosis in swine. *Veterinaria Italia*, 48(2), 133-56.

DONEV, D. 2010. Brucellosis control and eradication in the south eastern European countries: Current status and perspective strategies. *Macedonia Journal of Medical Science*, 3, 220. DOI: 10.3889/MJMS.1857-5773.2010.0134.

DORNELES, E.M., LIMA, K.G., TEIXEIRA-CARVALHO, A., ARAUJO, S.M., MARTINS-FILHO, A.O., SRIRANGANATHAN, N., AL QUBLAN, H., HEINEMANN, B.M. & LAGE, P.A. 2015. Immune response of calves vaccinated with *Brucella abortus* S19 or RB51 and revaccinated with RB51. *PLoS ONE*, 10, e0136696. DOI: 10.1371/journal.pone.0136696.

DUCROTOY, M.J., CONDE-ÁLVAREZ, R., BLASCO, J.M. & MORIYÓN, I. 2016. A review of the basis of the immunological diagnosis of ruminant brucellosis. *Veterinary Immunology and Immunopathology*, 171, 81-102. DOI:10.1016/j.vetimm.2016.02.002.

DUCROTOY, M., BERTU, W. J., MATOPE, G., CADMUS, S., CONDE-ÁLVAREZ, R., GUSI, A. M., WELBURN, S., OCHOLI, R., BLASCO, J.M. & MORIYÓN, I. 2017. Brucellosis in sub-Saharan Africa: Current challenges for management, diagnosis, and control. *Acta Tropica*, 165, 179– 193. DOI: 10.1016/j.actatropica.2015.10.023.

DUFFIELD, B.J., STREETEN, T.A. & SPINKS, G.A. 1984. Isolation of *Brucella abortus* from supramammary lymph nodes of cattle from infected herds vaccinated with low dose strain 19. *Australian Veterinary Journal*, 61, 411–412.

ERSOY, Y., SONMEZ, E. & TEVfiK, R.M. 2005. Comparison of three different combination therapies in the treatment of human brucellosis. *Tropical Doctor*, 35, 210–212. DOI: 10.1258/004947505774938765.

EU. 2001. Brucellosis in sheep and goats (*Brucella melitensis*), Health and Consumer Protection Directorate General, European Union. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out59_en.pdf. *Accessed September 23, 2018*.

FALLATAH, S.M., ODULOJU, J.S., AL-DUSARI, N.S. & FAKUNLE, M.Y. 2005. Human brucellosis in Northern Saudi Arabia. *Saudi Medical Journal*, 26, 1562–1566.

FAO/WHO. 1986. Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on brucellosis. World Health Organization Technical Report Series, 740, 1-132. Sixth Report. WHO, Geneva, Switzerland. https://apps.who.int/iris/handle/10665/40202.

FAO. 2003. Guidelines for coordinated animal and human brucellosis

Surveillance. FAO Animal Production and Health Paper 156, Rome, Italy. http://www.fao.org/3/y4723e/y4723e00.htm.

FAO. 2006. Brucellosis in humans and animals. Food and Agriculture Organisation, Rome, Italy. https://apps.who.int/iris/handle/10665/43597.

FAO, 2010. *Brucella melitensis* in Eurasia and the Middle East. FAO Animal Production and Health Proceedings. No. 10. Rome, Italy. http://www.fao.org/3/i1402e/i1402e00.htm.

FENSTERBANK, R. 1987. Comprehensive report. Brucellosis in cattle, sheep and goats: diagnosis control and vaccination. *Technical Series, Office International des épizooties*, 6, 9-35.

FERREIRA, A.C., CHAMBEL, L., TENREIRO, T., CARDOSO, R., FLOR, L., DIAS, I.T., PACHECO, T., GARIN-BASTUJI, B., LE FLÈCHE, P., VERGNAUD, G., TENREIRO, R. & CORRÊA DE SÁ, M.I. 2012. MLVA16 typing of Portuguese human and animal *Brucella melitensis* and *Brucella abortus* isolates. *PLoS ONE*, 7(8), e42514. DOI: 10.1371/journal.pone.0042514.

FOSTER, G., OSTERMAN, B.S., GODFROID, J., JACQUES, I. & CLOECKAERT, A. 2007. *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. *International Journal of Systematic and Evolutionary Microbiology*, 57, 2688–2693. DOI: 10.1099/ijs.0.65269-0.

FRANC, K.A., KRECEK, C.R., HASLER, N.B. & ARENAS-GAMBOA, M.A. 2018. Brucellosis remains a neglected disease in the developing world: A call for interdisciplinary action. *BMC Public Health*, 18, 125. DOI: 10.1186/s12889-017-5016-y.

FRANCO, M.P., MULDER, M., GILMAN, R.H. & SMITS, H.L. 2007. Human brucellosis. *Lancet Infectious Diseases*, 7(12), 775–786. DOI: 10.1016/S1473-3099(07)70286-4.

FROLICH, K., THIEDE, S., KOZIKOWSKI, T. & JAKOB, W. 2002. A review of mutual transmission of important infectious diseases between livestock and wildlife in Europe. *Annals of the New York Academy of Sciences*, 969, 4–13. DOI: 10.1111/j.1749-6632.2002.tb04343.x.

FSAI. 2009. Health risks from unpasteurized milk. http://www.fsai.ie. Accessed September 25, 2019

GALL, D. & NIELSEN, K. 2004. Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. *Revue Scientifique et Technique (International Office of Epizootics*), 23(3), 989-1002.

GARIN-BASTUJI, B., BLASCO, J.M., GRAYON, M. & VERGER, J.M. 1998. *Brucella melitensis* infection in sheep: present and future. *Veterinary Research,* 29, 255-274.

GARIN-BASTUJI, B., HUMMEL, N., GERBIER, G., CAU, C., POUILLOT, R., DA COSTA, M. & FONTAINE, J.J. 1999. Nonspecific serological reactions in the diagnosis of bovine brucellosis: experimental oral infection of cattle with repeated doses of *Yersinia enterocolitica* O:9. *Veterinary Microbiology*, 66(3), 223-33. DOI: 10.1016/s0378-1135(99)00010-3.

GAROFOLO, G., DI GIANNATALE, E., DE MASSIS, F., ZILLI, K., ANCORA, M., CAMMÀ, C., CALISTRI, P. & FOSTER, J.T. 2013. Investigating genetic diversity of *Brucella abortus* and *Brucella melitensis* in Italy with MLVA-16. *Infection, Genetics and Evolution*, 19, 59-70. DOI: 10.1016/j.meegid.2013.06.021.

GAROFOLO, G., FASANELLA, A., DI GIANNATALE, E., PLATONE, I., SACCHINI, L., PERSIANI, T., BOSKANI, T., RIZZARDI, K. & WAHAB, T. 2016. Cases of human brucellosis in Sweden linked to Middle East and Africa. *BMC Research Notes*, 9, 277. DOI: 10.1186/s13104-016-2074-7.

GIANNACOPOULOS, I., ELIOPOULOU, M.I., ZIAMBARAS, T. & PAPANASTASIOU, D.A. 2002. Transplacentally transmitted congenital brucellosis due to *Brucella abortus*. *Journal of Infection*, 45, 209–210. DOI: 10.1016/s0163-4453(02)91043-1.

GODFROID, J. 2002. Brucellosis in wildlife. *Revue Scientifique et Technique-Office International des Epizooties,* 21(2), 277–286. DOI: 10.20506/rst.21.2.1333.

GODFROID, J., GARIN-BASTUJI, B., BLASCO, J.M., THOMSON, J. AND THOEN, C.O. 2004. *Brucella melitensis* infection. In J.A.W. COETZER, G.R. THOMSON & R.C. TUSTIN (eds.), Infectious Diseases of livestock, pp. 1535-1541, Oxford University Press, Cape Town.

GODFROID, J., CLOECKAERT, A., LIAUTARD, J.P., KOHLER, S., FRETIN, D.,

WALRAVENS, K., GARIN-BASTUJI, B. & LETESSON, J.J. 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Veterinary Research*, 36(3), 313–326. DOI: 10.1051/vetres:2005003.

GODFROID, J., NIELSEN, K. & SAEGERMAN, C. 2010. Diagnosis of brucellosis in livestock and wildlife. *Croatian Medical Journal*, 51(4), 296–305. DOI: 10.3325/cmj.2010.51.296.

GODFROID, J., SCHOLZ, C.H., BARBIER, T., NICOLAS, C., WATTIAU, P., FRETIN, D., WHATMORE, M.A., CLOECKAERT, A., BLASCO, M.J., MORIYON, I., SAEGERMAN, C., MUMA, J.B., AL DAHOUK, S., NEUBAUER, H. & LETESSON, J.J. 2011. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive Veterinary Medicine* 102, 118–131. DOI: 10.1016/j.prevetmed.2011.04.007.

GODFROID, J., GARIN-BASTUJI, B., SAEGERMAN, C. & BLASCO, J.M. 2013. Brucellosis in terrestrial wildlife. *Revue Scientifique et Technique (International Office of Epizootics),* 32, 27–42. DOI: 10.20506/rst.32.1.2180.

GODFROID, J., DE BOLLE, X., ROOP, R.M., O'CALLAGHAN, D., TSOLIS, R.M., BALDWIN, C., SANTOS, R.L., MCGIVEN, J., OLSEN, S., NYMO, I.H., LARSEN, A., AL DAHOUK, S. & LETESSON, J.J. 2014. The quest for a true One Health perspective of brucellosis. *Revue Scientifique et Technique (International Office of Epizootics*), 33 (2), 521-538. DOI: 10.20506/rst.33.2.2290.

GODFROID, J. 2017. Brucellosis in livestock and wildlife: Zoonotic diseases without pandemic potential in need of innovative one health approaches. *Archives of Public Health*, 75, 34. DOI: 10.1186/s13690-017-0207-7.

GODFROID, J. 2018. *Brucella* spp. at the wildlife-livestock interface: An evolutionary trajectory through a livestock-to-wildlife "Host Jump"? *Veterinary Science*, 5, 81. DOI: 10.3390/vetsci5030081.

GÓMEZ, M., NIETO, J., ROSA, C., GEIJO, P., ESCRIBANO, M., MUNOZ, A. & LOPEZ, C. 2008. Evaluation of seven tests for diagnosis of human brucellosis in an area where the disease is endemic. *Clinical and Vaccine Immunology,* 15, 1031–1033. DOI: 10.1128/CVI.00424-07.

GRACE, D. 2015. Food safety in low- and middle-income countries. *International*

Journal of Environmental Research and Public Health, 12(9), 10490–10507. DOI: 10.3390/ijerph120910490.

GREINER, M., VERLOO, D. & DE MASSIS, F. 2009. Meta-analytical equivalence studies on diagnostic tests for bovine brucellosis allowing assessment of a test against a group of comparative tests. *Preventive Veterinary Medicine*, 92(4), 373-381. DOI: 10.1016/j.prevetmed.2009.07.014.

HALD, T., ASPINALL, W., DEVLEESSCHAUWER, B., COOKE, R., CORRIGAN, T., HAVELAAR, A.H., GIBB, H.J., TORGERSON, P.R., KIRK, M.D., ANGULO, F.J., LAKE, R.J., SPEYBROECK, N. & HOFFMANN, S. 2016. World Health Organization estimates of the relative contributions of food to the burden of disease due to selected foodborne hazards: A structured expert elicitation. *PLoS ONE*, 11, e0145839. DOI: 10.1371/journal.pone.0145839.

HALLING, S.M., TATUM, F.M. & BRICKER, B.J. 1993. Sequence and characterization of an insertion sequence, IS711, from *Brucella ovis*. *Gene*, 133, 123-127. DOI: 10.1016/0378-1119(93)90236-v.

HALLING, S.M., PETERSON-BURCH, B.D., BRICKER, B.J., ZUERNER, R.L., QING, Z. & LI, L.L. 2005. Completion of the genome sequence of Brucella abortus and comparison to the highly similar genomes of *Brucella melitensis* and *Brucella suis*. *Journal of Bacteriology*, 187, 2715–26. DOI: 10.1128/JB.187.8.2715-2726.2005.

HARTADY, T., SAAD, Z.M., BEJO, K.S. & SALISI, S.M. 2014. Clinical human brucellosis in Malaysia: a case report. *Asian Pacific Journal of Tropical Disease*, 4, 150–153. DOI: 10.1016/S2222-1808(14)60332-7.

HESTERBERG, U.W., BAGNALL, R., PERRETT, K., BOSCH, B., HORNER, R. & GUMMOW, B. 2008. A serological prevalence survey of *Brucella abortus* in cattle of rural communities in the province of KwaZulu-Natal, South Africa. *Journal of the South African Veterinary Association* 79, 15–18. DOI: 10.4102/jsava.v79i1.234.

HILLIS, D. M. & DIXON, M.T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *Quarterly Review of Biology*, 66, 411–452. DOI: 10.1086/417338.

HOWE, K.S., HÄSLER, B. & STÄRK, K.D.C. 2013. Economic principles for resource

allocation decisions at national level to mitigate the effects of disease in farm animal populations. *Epidemiology and Infection*, 141(1), 91–101. DOI: 10.1002/9781118960608.gbm00807.

HUBER, B., SCHOLZ, H.C., LUCERO, N. & BUSSE, H.J. 2009. Development of a PCR assay for typing and subtyping of *Brucella* species. *International Journal of Medical Microbiology*, 299, 563–573. DOI: 10.1016/j.ijmm.2009.05.002.

HULL, N.C. & SCHUMAKER, B.A. 2018. Comparisons of brucellosis between human and veterinary medicine. *Infection Ecology and Epidemiology*, 8(1), 1500846. DOI: 10.1080/20008686.2018.1500846.

IVANOV, A.V., SALMAKOV, K.M., OLSEN, S.C. & PLUMB, G.E. 2011. A live vaccine from *Brucella abortus* strain 82 for control of cattle brucellosis in the Russian Federation. *Animal Health Research Reviews*, 12(1), 113-21. DOI: 10.1017/S1466252311000028.

IBIRONKE, A.A., MCCRINDLE, C.M., FASINA, F.O. & GODFROID, J. 2008. Evaluation of problems and possible solutions linked to the surveillance and control of bovine brucellosis in sub-Saharan Africa, with special emphasis on Nigeria. *Veterinaria Italiana*, 44, 549– 556.

JIANG, H., DONG, H., PENG, X., FENG, Y., ZHU, L., NIU, K., PENG, Y., FAN, H. & DING, J. 2018. Transcriptome analysis of gene expression profiling of infected macrophages between *Brucella suis* 1330 and live attenuated vaccine strain S2 displays mechanistic implication for regulation of virulence. *Microbial Pathogenesis*, 119, 241–247. DOI: 10.1016/j.micpath.2018.04.003.

JIMÉNEZ DE BAGÜES, M.P., MARÍN, C. & BLASCO, J.M. 1991. Effect of antibiotic therapy and strain 19 vaccination on the spread of *Brucella melitensis* within an infected dairy herd. *Preventive Veterinary Medicine* 11, 17–24. DOI: 10.1016/S0167-5877(05)80041-8.

JIMÉNEZ DE BAGÜES, M.P., TERRAZA, A., GROSS, A. & DORNAND, J. 2004. Different responses of macrophages to smooth and rough *Brucella* spp.: relationship to virulence. *Infection and Immunity*, 72, 2429-2433. DOI: 10.1128/IAI.72.4.2429-2433.2004.

KAHN, C.M. 2008. Merck Veterinary Manual, 9th edn. Merck and Co. Inc., Whitehouse Station, New Jersey.

KHAN, M.Z. & ZAHOOR, M. 2018. An overview of brucellosis in cattle and humans,

and its serological and molecular diagnosis in control strategies. *Tropical Medicine and Infectious Diseases*, 3, 65. DOI: 10.3390/tropicalmed3020065.

KARABAY, O., SENCAN, I., KAYAS, D. & SAHIN, I. 2004. Ofloxacin plus rifampicin versus doxycycline plus rifampicin in the treatment of brucellosis: A randomized clinical trial. *BMC Infectious Diseases*, 4, 18. DOI: 10.1186/1471-2334-4-18.

KATO, Y., MASUDA, G., ITODA, I., IMAMURA, A., AJISAWA, A. & NEGISHI, M. 2007. Brucellosis in a returned traveler and his wife: probable person-to-person transmission of *Brucella melitensis*. *Journal of Travel Medicine*, 14(5), 343-5. DOI: 10.1111/j.1708-8305.2007.00139.x.

KAYA, S., ELALDI, N., DEVECI, O., ESKAZAN, E.A., BEKCIBASI, M. & HOSOGLU, S. 2018. Cytopenia in adult brucellosis patients. *Indian Journal of Medical Research*, 147, 73–80. DOI: 10.4103/ijmr.IJMR_542_15.

KOLAR, J., 1984. Diagnosis and control of brucellosis in small ruminants. *Preventive Veterinary Medicine,* 2, 215-225. DOI: 10.1016/0167-5877(84)90065-5.

KOLO, F.B., ADESIYUN, A.A., FASINA, F.O., KATSANDE, C.T., DOGONYARO, B.B., POTTS, A., MATLE, I., GELAW, A.K. & VAN HEERDEN, H. 2019. Seroprevalence and characterization of *Brucella* species in cattle slaughtered at Gauteng abattoirs, South Africa. *Veterinary Medicine and Science*, 5 (4), 545–555. DOI: 10.1002/vms3.190.

KOSE, S., SERIN SENGER, S., AKKOCLU, G., KUZUCU, L., ULU, Y., ERSAN, G. & OGUZ, F. 2014. Clinical manifestations, complications, and treatment of brucellosis: Evaluation of 72 cases. *Turkish Journal of Medical Science*, 44, 220–223. DOI: 10.3906/sag-1112-34.

KOTTON, C.N. 2007. Zoonoses in solid-organ and hematopoietic stem cell transplant recipients. *Clinical and Infectious Disease*, 44(6), 857-66. DOI: 10.1086/511859.

KOUBA, V. 2003. A method of accelerated eradication of bovine brucellosis in the Czech Republic. *Revue Scientifique et Technique de l'OIE*, 22, 1003–1012. DOI: 10.20506/rst.22.3.1447.

KÜSTNER, H.G.V. 1985. Brucellosis in the eastern Orange Free State. *Epidemiological Comments*, 12(3), 2-23.

LEAL-KLEVEZAS, D.S., MARTÍNEZ-VÁZQUEZ, I.O., LÓPEZ-MERINO, A. &

MARTÍNEZ-SORIANO, J.P. 1995. Single-Step PCR for detection of *Brucella* spp. from blood and milk of infected animals. *Journal of Clinical Microbiology*, 33(12), 3087–3090. DOI:10.1128/JCM.33.12.3087-3090.1995.

LEE, S., HWANG, K.J., PARK, M.Y., HWANG, S.D., CHAI, H.Y., CHU, H. & PARK, S.H. 2013. Evaluation and selection of multilocus variable-number tandem-repeat analysis primers for genotyping *Brucella abortus* biovar 1 isolated from human patients. *Osong Public Health and Research Perspectives*, 4, 265-270. DOI: 10.1016/j.phrp.2013.09.005.

LE FLÈCHE, P., JACQUES, I., GRAYON, M., AL DAHOUK, S., BOUCHON, P., DENOEUD, F., NÖCKLER, K., NEUBAUER, H., GUILLOTEAU, L.A. & VERGNAUD, G. 2006. Evaluation and selection of tandem repeat loci for a *Brucella* MLVA typing assay. *BMC Microbiology*, 6, 9. DOI: 10.1186/1471-2180-6-9.

LEDWABA, B., MAFOFO, J. & VAN HEERDEN, H. 2014. Genome sequences of *Brucella abortus* and *Brucella suis* strains isolated from bovine in Zimbabwe. *Genome Announcement*, 2(5), e01063-14. DOI: 10.1128/genomeA.01063-14.

LEDWABA, M.B., GOMO, C., LEKOTA, K.E., LE FLÈCHE, P., HASSIM, A., VERGNAUD, G. & VAN HEERDEN, H. 2019. Molecular characterization of *Brucella* species from Zimbabwe. *PLoS Neglected Tropical Diseases*, 13(5), e0007311. DOI: 10.1371/journal.pntd.0007311.

LINDAHL, E., SATTOROV, N., BOQVIST, S., SATTORI, I. & MAGNUSSON, U. 2014. Seropositivity and risk factors for *Brucella* in dairy cows in urban and peri-urban small-scale farming in Tajikistan. *Tropical Animal Health and Production*, 46, 563–569. DOI: 10.1007/s11250-013-0534-9.

LI, M-T., SUN, G-Q., ZHANG, W-Y. & JIN, Z. 2017. Model-based evaluation of strategies to control brucellosis in China. *International Journal of Environmental Research and Public Health*, 14(3), 295. DOI: 10.3390/ijerph14030295.

LÓPEZ-GOÑI, I., GARCÍA-YOLDI, D., MARÍN, C.M., DE MIGUEL, M.J., MUÑOZ, P.M., BLASCO, J.M., JACQUES, I., GRAYON, M., CLOECKAERT, A., FERREIRA, A.C., CARDOSO, R., CORRÊA DE SÁ, M.I., WALRAVENS, K., ALBERT, D. & GARIN-BASTUJI, B. 2008. Evaluation of a multiplex PCR

assay (Bruce-ladder) for molecular typing of all *Brucella* species, including the vaccine strains. *Journal of Clinical Microbiology*, 46(10), 3484-3487. DOI: 10.1128/JCM.00837-08.

LÓPEZ–GOÑI, I., GARCÍA–YOLDI, D., MARÍN, C.M., DE MIGUEL, M.J., BARQUERO-CALVO, E., GUZMÁN-VERRI, C., ALBERT, D. & GARIN-BASTUJI, B. 2011. New Bruce-ladder multiplex PCR assay for the biovar typing of *Brucella suis* and the discrimination of *Brucella suis* and *Brucella canis*. *Veterinary Microbiology*, 154, 152–155. DOI: 10.1016/j.vetmic.2011.06.035.

LUCERO, N.E., ESCOBAR, G.I., AYALA, S.M., SILVA PAULO P. & NIELSEN, K. 2003. Fluorescence polarization assay for diagnosis of human brucellosis. *Journal of Medical Microbiology*, 52, 883-887. DOI: 10.1099/jmm.0.05217-0.

LUCERO, N.E., ESCOBAR, I.G., AYALA, M.S. & JACOB, N. 2005. Diagnosis of human brucellosis caused by *Brucella canis*. *Journal of Medical Microbiology*, 54, 457–461. DOI: 10.1099/jmm.0.45927-0.

LUCERO, N.E., AYALA, S.M., ESCOBAR, G.I. & JACOB, N.R. 2008. *Brucella* isolated in humans and animals in Latin America from 1968 to 2006. *Epidemiology and Infection*, 136(4), 496–503. DOI: 10.1017/S0950268807008795.

LUCERO, N.E., CORAZZA, R., ALMUZARA, M.N., REYNES, E., ESCOBAR, G.I., BOERI, E. & AYALA, S.M. 2010: Human *Brucella canis* outbreak linked to infection in dogs. *Epidemiology and infection*, 138, 280-285. DOI: 10.1017/S0950268809990525.

MADZINGIRA, O. & MCCRINDLE, C. 2014. Prevalence of *Brucella* antibodies in sheep and springbok (*Antidorcas marsupialis*) reared together in the Karas region, Namibia, *Bulletin of Animal Health and Production in Africa*, 62, 299-306.

MADZINGIRA, O. & MCCRINDLE, C. 2015. Retrospective analysis of the prevalence of *Brucella* antibodies in sheep in the Karas Region of Namibia. *Tropical Animal Health and Production*, 47, 1117. DOI: 10.1007/s11250-015-0838-z.

MADZINGIRA, O. & MCCRINDLE, C. 2016. A questionnaire survey of risk factors of

brucellosis on mixed sheep and springbok (*Antidorcas marsupialis*) farms. *International Science and Technology Journal of Namibia*, 8, 43-49.

MAGWEDERE, K., BISHI, A., TJIPURA-ZAIRE, G., EBERLE, G., HEMBERGER, Y., HOFFMAN, L.C. & DZIVA, F. 2011. Brucellae through the food chain: the role of sheep, goats and springbok (*Antidorcus marsupialis*) as sources of human infection in Namibia. *Journal of the South African Veterinary Association*, 82, 205-212. DOI: 10.4102/jsava.v82i4.75.

MAINAR-JAIME, R.C. & VÁZQUEZ-BOLAND, J.A. 1999. Associations of veterinary services and farmer characteristics with the prevalence of brucellosis and border disease in small ruminants in Spain. *Preventive Veterinary Medicine*, 40(3-4), 193-205. DOI: 10.1016/s0167-5877(99)00027-6.

MAKITA, K., FEVRE, E.M, WAISWA, C., KABOYO, W., DE CLARE BRONSVOORT, M.B., EISLER, C.M. & WELBURN, C.S. 2008. Human brucellosis in urban and peri-urban areas of Kampala, Uganda. *Annals of the New York Academy of Sciences*, 1149, 309–311. DOI: 10.1196/annals.1428.015.

MAKITA, K., FÈVRE, E.M., WAISWA, C., EISLER, M.C. & WELLBURN, S.C. 2010. How human brucellosis incidence in urban Kampala can be reduced most efficiently? A stochastic risk assessment of informally-marketed milk. *PLoS ONE*, 5(12), e14188. DOI: 10.1371/journal.pone.0014188.

MAKITA, K., FE`VRE, M.E., WAISWA, C., EISLER, M., THRUSFIELD, M. & WELLBURN, S.C., 2011. Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. *BMC Veterinary Research*, 7, 60. DOI: 10.1186/1746-6148-7-60.

MANGEN, M-J, OTTE, J., PFEIFFER, D. & CHILONDA, P. 2002. Bovine brucellosis in Sub-Saharan Africa: estimation of sero-prevalence and impact on meat and milk offtake potential. Food and Agricultural Organisation, Rome, Italy.

MANTUR, B.G., BIRADAR, S.M., BIDRI, C.R., MULIMANI, S.M., VEERAPPA, P., KARIHOLU, P., PATIL, B.S. & MANGALGI, S.S. 2006. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. *Journal of Medical Microbiology*, 55, 897–903. DOI: 10.1099/jmm.0.46097-0.

MARCOTTY, T., MATTHYS, F., GODFROID, J., RIGOUTS, L., AMENI, G., VAN

PITTIUS, N.G., KAZWALA, R., MUMA, J., VAN HELDEN, P., WALRAVENS, K., DE KLERK, L.M., GEOGHEGAN, C., MBOTHA, D., OTTE, M., AMENU, K., SAMRA, N.A., BOTHA, C., EKRON, M., JENKINS, A., JORI, F., KRIEK, N., MCCRINDLE, C., MICHEL, A., MORAR, D., ROGER, F. & THYS, E. 2009. Zoonotic tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health issues? The outcomes of a multi-disciplinary workshop. *Annals of Tropical Medicine and Parasitology*, 103(5), 401–411. DOI: 10.1179/136485909X451771.

MARTINS, H., GARIN-BASTUJI, B., LIMA, F., FLOR, L., PINA FONSECA, A. & BOINAS, F. 2009. Eradication of bovine brucellosis in the Azores, Portugal—Outcome of a 5-year programme (2002–2007) based on test-and-slaughter and RB51 vaccination. *Preventive Veterinary Medicine*, 90, 80–89. DOI: 10.1016/j.prevetmed.2009.04.002.

MATOPE, G., MUMA, J.B., TOFT, N., GORI, E., LUND, A., NIELSEN, K. & SKJERVE, E. 2011. Evaluation of sensitivity and specificity of RBT, c-ELISA and fluorescence polarisation assay for diagnosis of brucellosis in cattle using latent class analysis. *Veterinary Immunology and Immunopathology*, 141(1-2), 58-63. DOI: 10.1016/j.vetimm.2011.02.005.

MAYFIELD, J.E., BRICKER, B.J., GODFREY, H., CROSBY, R.M., KNIGHT, D.J., HALLING, S.M., BALINSKY, D. & TABATABAI, L.B. 1988. The cloning, expression, and nucleotide sequence of a gene coding for an immunogenic *Brucella abortus* protein. *Gene*, 63 (1), 1-9. DOI: 10.1016/0378-1119(88)90540-9.

MCDERMOTT, J.J. & ARIMI, S.M. 2002. Brucellosis in sub-Saharan Africa: epidemiology, control, and impact. *Veterinary Microbiology*, 90(1–4), 111–134. DOI: 10.1016/s0378-1135(02)00249-3.

MCDERMOTT, J.D., GRACE, D. & ZINSSTAG, J. 2013. Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique-Office International des Epizooties*, 32(1), 249–61. DOI: 10.20506/rst.32.1.2197.

MCDONALD, W.L., JAMALUDIN, R., MACKERETH, G., HANSEN, M., HUMPHREY, S., SHORT, P., TAYLOR, T., SWINGLER, J., DAWSON, C.E., WHATMORE, A.M., STUBBERFIELD, E., PERRETT, L.L. & SIMMONS, G. 2006.

Characterization of a *Brucella* sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. *Journal of Clinical Microbiology*, 44, 4363-4370. DOI: 10.1128/JCM.00680-06.

MCGIVEN, J.A., TUCKER, J.D., PERRETT, L.L., STACK, J.A., BREW S.D. & MACMILLAN, A.P. 2003. Validation of FPA and cELISA for the detection of antibodies to *Brucella abortus* in cattle sera and comparison to SAT, CFT, and iELISA. *Journal of Immunological Methods*, 278, 171-178. DOI: 10.1016/s0022-1759(03)00201-1.

MCGIVEN, J., NICOLA, A., COMMANDER, N., DUNCOMBE, L., TAYLOR, A.V., VILLARI, S., DAINTY, A., THIRLWALL, R., BOUZELMAT, N., PERRETT, L., BREW, S. & STACK, J. 2012. An evaluation of the capability of existing and novel serodiagnostic methods for porcine brucellosis to reduce false positive serological reactions. *Veterinary Microbiology*, 160 (3–4), 378–386. DOI: 10.1016/j.vetmic.2012.06.007.

MCGIVEN, J.A. 2013. New developments in the immunodiagnosis of brucellosis in livestock and wildlife. In Brucellosis: recent developments towards 'One Health' (Plumb, G., Olsen, S and Pappas, G. eds). *Revue Scientifique et Technique (International Office of Epizootics*), 32 (1), 163–176. DOI: 10.20506/rst.32.1.2205.

MEGERSA, B., BIFFA, D., ABUNNA, F., REGASSA, A., GODFROID, J. & SKJERVE, E. 2011. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Tropical Animal Health and Production*, 43, 651-656. DOI: 10.1007/s11250-010-9748-2.

MENG, F., PAN, X. & TONG, W. 2018. Rifampicin versus streptomycin for brucellosis treatment in humans: A meta-analysis of randomized controlled trials. *PLoS ONE,* 13, e0191993. DOI: 10.1371/journal.pone.0191993.

MESNER, O., RIESENBERG, K., BILIARM, N., BORSTEIN, E., BOUHNIK, L., PELED, N. & YAGUPSKY, P. 2007. The many faces of human-to-human transmission of brucellosis: congenital infection and outbreak of nosocomial disease related to an unrecognized clinical case. *Clinical Infectious Diseases,* 45, e135-40. DOI: 10.1086/523726.

MILI, N., AUCKENTHALER, R. & NICOD, L.P. 1993. Chronic *Brucella* empyema.

Chest, 103, 620–621. DOI: 10.1378/chest.103.2.620.

MILWARD, F.W., NICOLETTI, P. & HOFFMAN, E. 1984. Effectiveness of various therapeutic regimens for bovine brucellosis. *American Journal of Veterinary Research*, 45, 1825-1828.

MINAS, M., A. MINAS, K. GOURGULIANIS & A. STOURNARA, 2007a. Epidemiological and clinical aspects of human brucellosis in Central Greece. *Japan Journal of Infectious Diseases*, 60, 362-366.

MINAS, A., STOURNARA, A., MINAS, M., STACK, J., PETRIDOU, E., CHRISTODOULOPOULOS, G. & KRIKELIS, V. 2007b. Validation of a fluorescence polarization assay (FPA) performed in microplates and comparison with other tests used for diagnosing *B. melitensis* infection in sheep and goats. *Journal of Immunological Methods*, 320(1-2), 94-103. DOI: 10.1016/j.jim.2006.12.008.

MIRANDA, K.L., DORNELES, M.E., PAULETTI, B.R., POESTER, P.F. & LAGE, P.A. 2015. *Brucella abortus* S19 and RB51 vaccine immunogenicity test: Evaluation of three mice (BALB/c, Swiss and CD-1) and two challenge strains (544 and 2308). *Vaccine*, 33, 507–511. DOI: 10.1016/j.vaccine.2014.11.056.

MOHAN, K., MAKAYA, P.V., MUVAVARIRWA, P., MATOPE, G., MAHEMBE, E. & PAWANDIWA, A. 1996. Brucellosis surveillance and control in Zimbabwe: bacteriological and serological investigation in dairy herds. *Onderstepoort Journal of Veterinary Research*, 63, 47–51.

MORE, S.J., RADUNZ, B. & GLANVILLE, R.J. 2015. Lessons learned during the successful eradication of bovine tuberculosis from Australia. *Veterinary Record*, 177, 224– 232. DOI: 10.1136/vr.103163.

MORENO, S., ARIZA, J., ESPINOSA, F.J., PODZAMCZER, D., MIRÓ, J.M., RIVERO, A., RODRÍGUEZ-ZAPATA, M., ARRIZABALAGA, J., MATEOS, R. & HERRERO, F. 1998. Brucellosis in patients infected with the human immunodeficiency virus. *European Journal of Clinical Microbiology and Infectious Disease*, 17(5), 319-26. DOI: 10.1007/bf01709454.

MORENO, E., CLOECKAERT, A. & MORIYON, I. 2002. *Brucella* evolution and taxonomy. *Veterinary Microbiology*, 90, 209–227. DOI: 10.1016/s0378-1135(02)00210-9.

MORENO E. & MORIYÓN I. 2006. The genus *Brucella*. In: *The Prokaryotes* Vol. 5

Part 1, Section 31, DWORKIN, M., FALKOW, S., ROSENBERG, E., SCHLEIFER, K.-H., STACKEBRANT, E. (eds), New York: Springer-Verlag, 315–456.

MORENO, E. 2014. Retrospective and prospective perspectives on zoonotic brucellosis. *Frontiers in Microbiology*, 5, 213. DOI: 10.3389/fmicb.2014.00213.

MORIYÓN, I., GRILLÓ, M.J, MONREAL, D., GONZALEZ, D., MARÍN, C.M., LÓPEZ-GOÑI, I., MAINAR-JAIME, R.C., MORENO, E. & BLASCO, J.M. 2004. Rough vaccines in animal brucellosis: structural and genetic basis and present status. *Veterinary Research*, 35, 1–38. DOI: 10.1051/vetres:2003037.

MUMA, J.B., SAMUI, K.L., SIAMUDAALA, V.M., OLOYA, J., MATOPE, G., OMER, M.K., MUNYEME, M., MUBITA, C. & SKJERVE, E. 2006. Prevalence of antibodies to *Brucella* spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock–wildlife interface areas of Zambia. *Tropical Animal Health and Production*, 38, 195-206. DOI: 10.1007/s11250-006-4320-9.

MUMA, J.B., SAMUI, K.L., OLOYA, J., MUNYEME, M. & SKJERVE, E. 2007. Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia. *Preventive Veterinary Medicine*, 80, 306-317. DOI: 10.1016/j.prevetmed.2007.03.003.

MUÑOZ, P.M., MARIN, C.M., MONREAL, D., GONZALEZ, D., GARIN-BASTUJI, B., DIAZ, R., MAINAR-JAIME, R.C., MORIYON, I. & BLASCO, J.M. 2005. Efficacy of several serological tests and antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to *Yersinia enterocolitica* O:9. *Clinical and Diagnostic Laboratory Immunology*, 12(1), 141-151. DOI: 10.1128/CDLI.12.1.141-151.2005.

MUSALLAM, I.I., ABO-SHEHADA, N.M., HEGAZY, M.Y., HOLT, R.H. & GUITIAN, J.F. 2016. Systematic review of brucellosis in the Middle East: Disease frequency in ruminants and humans and risk factors for human infection. *Epidemiology and Infection*, 144, 671–685. DOI: 10.1017/S0950268815002575.

NICOLETTI, P. 1980. The epidemiology of bovine brucellosis. *Advances in Veterinary Science and Comparative Medicine*, 24, 69.

NICOLETTI, P., MILWARD, F.W. & HOFFMAN, E. 1985. Efficacy of a long-acting

oxytetracycline alone or combined with streptomycin in the treatment of bovine brucellosis. *Journal of the American Veterinary Medical Association*, 187, 493-495.

NICOLETTI, P., LENK, R.P., POPESCU, M.C. & SWENSON, C.E. 1989. Efficacy of various treatment regimens, using liposomal streptomycin in cows with brucellosis. *American Journal of Veterinary Research*, 50, 1004-1007.

NICOLETTI, P. 1990. Vaccination. In: NIELSEN, K. & DUNCAN J.R. (eds.), Animal Brucellosis. CRC Press, Boca Raton, Florida.

NICOLETTI, P. 1993. Brucellosis. In: *Current Veterinary Therapy 3, Food Animal Practice*. HOWARD, J.L. (ed): W.B. Saunders Co., Philadelphia, pp 551-555.

NICOLETTI, P. 2010. Brucellosis: past, present, and future. *Prilozi*, 31(1), 21-32.

NIELSEN, K., GALL, D., JOLLEY, M., LEISHMAN, G., BALSEVICIUS, S., SMITH, P., NICOLETTI, P. & THOMAS, F. 1996. A homogeneous fluorescence polarization assay for detection of antibody to *Brucella abortus*. *Journal of Immunological Methods*, 195, 161-168. DOI: 10.1016/0022-1759(96)00116-0.

NIELSEN, K. 2002. Diagnosis of brucellosis by serology. *Veterinary Microbiology*, 90(1), 447–459. DOI: 10.1016/s0378-1135(02)00229-8.

NIELSEN, K., GALL, D., SMITH, P., BALSEVICIUS, S., GARRIDO, F., FERRER, M.D., BIANCIFIORI, F., DAJER, A., LUNA, E., SAMARTINO, L., BERMUDEZ, R., MORENO, F., RENTERIA T. & CORRAL, A. 2004. Comparison of serological tests for the detection of ovine and caprine antibody to *Brucella melitensis*. *Revue scientifique et technique (International Office of Epizootics)*, 23, 979-987. DOI: 10.20506/rst.23.3.1532.

NIELSEN, K., SMITH, P., YU W., NICOLETTI, P., JURGERSEN, G., STACK, J. & GODFROID, J. 2006. Serological discrimination by indirect enzyme immunoassay between the antibody response to *Brucella* sp. and *Yersinia enterocolitica* O: 9 in cattle and pigs. *Veterinary Immunology and Immunopathology*, 109, 69–78. DOI: 10.1016/j.vetimm.2005.07.025.

NIELSEN, K. & YU, W.L. 2010. Serological diagnosis of brucellosis. *Prilozi*, 31, 65-89. OBEREM, P., ODENDAAL, D., OBEREM, P.T., SNYMAN, M.G.S., LUDWIG, L. & MYNHARDT, H. 2006. Diseases and parasites of cattle, sheep and goats in South Africa, Afrivet Business Management, Pretoria.

OIE. 2018. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter

3.1.4. Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis) (infection with B. abortus, B. melitensis* and *B. suis*). http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf. *Accessed February 16, 2019*.

O'LEARY, S., SHEAHAN, M. & SWEENEY, T. 2006. *Brucella abortus* detection by PCR assay in blood, milk and lymph tissue of serologically positive cows. *Research in Veterinary Science,* 81, 170–176. DOI: 10.1016/j.rvsc.2005.12.001.

OCAMPO–SOSA, A.A., AGÜERO–BALBÍN, J. & GARCÍA–LOBO, J.M. 2005. Development of a new PCR assay to identify *Brucella abortus* biovars 5, 6 and 9 and the new subgroup 3b of biovar 3. *Veterinary Microbiology*, 110, 41–51. DOI: 10.1016/j.vetmic.2005.06.007.

OLSEN, S.C. & STOFFREGEN, W.S. 2005. Essential role of vaccines in brucellosis control and eradication programs for livestock. *Expert Review of Vaccines*, 4(6), 915-28. DOI: 10.1586/14760584.4.6.915.

OLSEN, S. & TATUM, F. 2010. Bovine brucellosis. *The Veterinary Clinics of North America: Food Animal Practice*, 26, 15-27. DOI: 10.1016/j.cvfa.2009.10.006.

OSORO, E.M., MUNYUA, P., OMULO, S., OGOLA, E., ADE, F., MBATHA, P., MBABU, M., NG'ANG'A, Z., KAIRU, S., MARITIM, M., THUMBI, S.M., BITEK, A., GAICHUGI, S., RUBIN, C., NJENGA, K. & GUERRA, M. 2015. Strong association between human and animal Brucella seropositivity in a linked study in Kenya, 2012-2013. *The American journal of Tropical Medicine and Hygiene*, *93*(2), 224–231. DOI: 10.4269/ajtmh.15-0113.

OSTERMAN, B. & I. MORIYON. 2006. Report of the International Committee on Systematics Prokaryotes, Subcommittee on the taxonomy of *Brucella,* Minutes of the meeting, 17 September 2003, Pamplona, Spain*. International Journal of Systematic and Evolutionary Microbiology*, 56, 1173-1175. DOI: 10.1099/ijs.0.64349-0.

PAPPAS, G., AKRITIDIS, N., BOSILKOVSKI, M. & TSIANOS, E. 2005. Brucellosis. *New England Journal of Medicine*, 352(22), 2325–2336. DOI: 10.1056/NEJMra050570.

PAPPAS, G., PAPADIMITRIOU, P., AKRITIDIS, N., CHRISTOU, L. & TSIANOS,

E.V. 2006. The new global map of human brucellosis. *Lancet Infectious Diseases*, 6(2), 91–9. DOI: 10.1016/S1473-3099(06)70382-6.

PAPPAS, G. 2010. The changing *Brucella* ecology: novel reservoirs, new threats. *International Journal of Antimicrobial Agents*, 36(Suppl 1), S8-S11. DOI: 10.1016/j.ijantimicag.2010.06.013.

PATTERSON, J.M. & DEYOE, B.L. 1976. Effect of physical properties of milk fat globules on *Brucella* ring test sensitivity. *Journal of Dairy Science*, 60, 851-856. DOI: 10.3168/jds.S0022-0302(77)83953-2.

PAULSEN, I.T., SESHADRI, R., NELSON, K.E., EISEN, J.A., HEIDELBERG, J.F., READ, T.D., DODSON, R.J., UMAYAM, L., BRINKAC, L.M., BEANAN, M.J., DAUGHERTY, S.C., DEBOY, R.T., DURKIN, A.S., KOLONAY, J.F., MADUPU, R., NELSON, W.C., AYODEJI, B., KRAUL, M., SHETTY, J., MALEK, J., VAN AKEN, S.E., RIEDMULLER, S., TETTELIN, H., GILL, S.R., WHITE, O., SALZBERG, S.L., HOOVER, D.L., LINDLER, L.E., HALLING, S.M., BOYLE, S.M & FRASER, C.M. 2002. The *Brucella suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts. *Proceedings of the National Academy of Sciences* USA, 99, 13148–53. DOI: 10.1073/pnas.192319099.

PAVADE, G., AWADA, L., HAMILTON, K. & SWAYNE, D.E. 2011. The influence of economic indicators, poultry density and the performance of veterinary services on the control of high pathogenicity avian influenza in poultry. *Revue Scientifique et Technique*, 30, 661– 671.

PEELMAN, J. & DEKEYSER, P. 1987. De verspreiding van de *Brucella* infectie bij vlaamse werknemers professioneel in contact met runderen. *Vlaams Diergeneeskundig Tijschrift*, 56:314-23.

PERRETT, L.L., MCGIVEN, J.A., BREW, S.D. & STACK, J.A. 2010. Evaluation of competitive ELISA for detection of antibodies to *Brucella* infection in domestic animals. *Croatian Medical Journal*, 51(4), 314-9. DOI: 10.3325/cmj.2010.51.314.

PÉREZ-SANCHO, M., GARCÍA-SECO, T., DOMÍNGUEZ, L. & ÁLVAREZ, J. 2015. Control of animal brucellosis — the most effective tool to prevent human brucellosis, Updates on brucellosis, Manal Mohammad Baddour, IntechOpen, DOI: 10.5772/61222. Available from:

https://www.intechopen.com/books/updates-on-brucellosis/control-of-animal-brucellosis-the-most-effective-tool-to-prevent-human-brucellosis.

PETZER, I.M., VAN STADEN, J.J. & GIESECKE, W.H. 1984. Tissue reactions and residues in slaughter cattle after administration of long-acting oxytetracycline formulations. *Journal of the South African Veterinary Association*, 55, 107–111.

POESTER, F.P., NIELSEN, K., SAMARTINO, L.E. & YU, W.L. 2010. Diagnosis of brucellosis. *Open Veterinary Science Journal*, 4, 46–60. DOI: 10.2174/1874318801004010046.

POUILLOT, R., GARIN–BASTUJI, B., GERBIER, G., COCHE, Y., CAU, C., DUFOUR, B. & MOUTOU, F. 1997. The brucellin skin test as a tool to differentiate false positive serological reactions in bovine brucellosis. *Veterinary Research*, 28, 365–374.

PRAUD, A., GIMENEZ, O., ZANELLA, G., DUFOUR, B., POZZI, N., ANTRAS, V., MEYER, L. & GARIN-BASTUJI, B. 2012. Estimation of sensitivity and specificity of five serological tests for the diagnosis of porcine brucellosis. *Preventive Veterinary Medicine*, 104, 94–100. DOI: 10.1016/j.prevetmed.2011.10.014.

QUEIPO-ORTUÑO, M.I., COLMENERO, J.D., REGUERA, J.M., GARCÍA-ORDOÑEZ, M.A., PACHÓN, M.E., GONZALEZ, M. & MORATA, P. 2005. Rapid diagnosis of human brucellosis by SYBR Green I-based real-time PCR assay and melting curve analysis in serum samples. *Clinical Microbiology and Infection*, 11(9), 713–718. DOI: 10.1111/j.1469-0691.2005.01202.x.

QUEIPO-ORTUÑO, M.I., COLMENERO, J.D., MUÑOZ, N., BAEZA, G., CLAVIJO, E. & MORATA, P. 2006. Rapid diagnosis of *Brucella* epididymo-orchitis by real-time polymerase chain reaction assay in urine samples. *Journal of Urology*, 176(5), 2290–2293. DOI: 10.1016/j.juro.2006.07.052.

QUEIPO-ORTUÑO, M.I., COLMENERO, J.D., MACIAS, M., BRAVO, M.J. & MORATA, P. 2008. Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. *Clinical and Vaccine Immunology*, 15(2), 293–296. DOI: 10.1128/CVI.00270-07.

QUEIPO-ORTUÑO, M.I., COLMENERO, J.D., BERMUDEZ, P., BRAVO, M.J. &

MORATA, P. 2009. Rapid differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis using a multiplex real-time PCR assay. *PLoS ONE,* 4(2), e4526. DOI: 10.1371/journal.pone.0004526.

RADOSTITS, O.M., GAY, C.C., BLOOD, C.D. & HINCHCLIFF, K.W. 2000. Veterinary Medicine. A textbook of the disease of cattle, sheep, pigs, goats and horses, 9th edition, (W.B. Saunders Company Ltd : New York).

RADWAN, A.I., BEKAIRI, S.I., AL-BOKMY, A.M., PRASAD, P.V.S., MOHAMED, E.M. & HUSSAIN, S.T. 1993. Successful therapeutic regimens for treating *Brucella melitensis* and *Brucella abortus* infections in cows. *Revue scientifique et technique (International Office of Epizootics)*, 12(3), 909-922. DOI: 10.20506/rst.12.3.729.

RAGAN, V.E. 2002. The animal and plant health inspection service (APHIS) brucellosis eradication program in the United States. *Veterinary Microbiology*, 90(1–4), 11– 18. DOI: 10.1016/s0378-1135(02)00240-7.

RAMIREZ-PFEIFFER, C., NIELSEN, K., MARIN-RICALDE, F., RODRIGUEZ-PADILLA, C. & GOMEZ-FLORES, R. 2006. Comparison of fluorescence polarization assay with card and complement fixation tests for the diagnosis of goat brucellosis in a high-prevalence area. *Veterinary Immunology and Immunopathology*, 110, 121-127. DOI: 10.1016/j.vetimm.2005.09.011.

RAMIREZ-PFEIFFER, C., DIAZ-APARICIO, E., GOMEZ-FLORES, R., RODRIGUEZ-PADILLA, C., MORALES LOREDO, A. & ALVAREZ-OJEDA, G. 2008. Use of the *Brucella melitensis* native hapten to diagnose brucellosis in goats by a rapid, simple, and specific fluorescence polarization assay. *Clinical and Vaccine Immunology*, 15(6), 911-915. DOI: 10.1128/CVI.00046-08.

REGUERA, J.M., ALARCON, A., MIRALLES, F., PACHON, J., JUAREZ, C. & COLMENERO, J.D. 2003. *Brucella* endocarditis: clinical, diagnostic, and therapeutic approach. *European Journal of Clinical Microbiology and Infectious Disease*, 22, 647-650. DOI: 10.1007/s10096-003-1026-z.

ROUSHAN M.R., AMIRI, M.J., LALY, A., MOSTAFAZADEH, A. & BIJANI, A. 2010. Follow-up standard agglutination and 2-mercaptoethanol tests in 175 clinically cured cases of human brucellosis. *International Journal of Infectious Diseases*, 14(3), e250–e253. DOI: 10.1016/j.ijid.2009.05.008.

ROY, S., MCELWAIN, T.F. & WAN, Y. 2011. A network control theory approach to

modelling and optimal control of zoonoses: Case study of brucellosis transmission in sub-Saharan Africa. *PLoS Neglected Tropical Diseases*, 5, e1259. DOI: 10.1371/journal.pntd.0001259.

RUIZ-MESA, J.D., SÁNCHEZ-GONZALEZ, J., REGUERA, J.M., MARTÍN, L., LOPEZ-PALMERO, S. & COLMENERO, J.D. 2005. Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. *Clinical Microbiology and Infection*, 11(3), 221–225. DOI: 10.1111/j.1469-0691.2004.01063.x.

RUPPANNER, R., MEYER, M.E., WILLEBERG, P. & BEHYMER, D.E. 1980. Comparison of the enzyme-linked immunosorbent assay with other tests for brucellosis, using sera from experimentally infected heifers. *American Journal for Veterinary Research*, 41, 1329-1332.

SALTOGLU, N., TASOVA, Y., INAL, S.A., SEKI, T. & AKSU, S.H. 2002. Efficacy of rifampicin plus doxycycline versus rifampicin plus quinolone in the treatment of brucellosis. *Saudi Medical Journal*, 23, 921–924.

SANOGO, M., ABATIH, E., THYS, E., FRETIN, D., BERKVENS, D. & SAEGERMAN, C. 2012. Risk factors associated with brucellosis seropositivity among cattle in the central savannah-forest area of Ivory Coast. *Preventive Veterinary Medicine*, 107, 51-56. DOI: 10.1016/j.prevetmed.2012.05.010.

SANOGO, M., THYS, E., ACHI, Y.L., FRETIN, D., MICHEL, P., ABATIH, E., BERKVENS, D. & SAEGERMAN, C. 2013. Bayesian estimation of the true prevalence, sensitivity, and specificity of the rose bengal and indirect ELISA tests in the diagnosis of bovine brucellosis. *Veterinary Journal*, 195(1), 114-120. DOI: 10.1016/j.tvjl.2012.06.007.

SANTOS, R.L., MARTINS, T.M., BORGES, Á.M. & PAIXÃO, T.A. 2013. Economic losses due to bovine brucellosis in Brazil. *Pesquisa Veterinária Brasileira*, 33, 759–764. DOI: 10.1590/S0100-736X2013000600012.

SANZ, C., SAEZ, L., ALVAREZ, J., CORTES, M., PEREIRA, G., REYES, A., RUBIO, F., MARTIN, J., GARCIA, N., DOMINGUEZ, L., HERMOSO-DE-MENDOZA, M. & HERMOSO-DE-MENDOZA, J. 2010. Mass vaccination as a complementary tool in the control of a severe outbreak of bovine brucellosis due to *Brucella abortus* in Extremadura, Spain. *Preventive Veterinary Medicine*, 97, 119–125. DOI: 10.1016/j.prevetmed.2010.08.003.

SATHYANARAYAN, M.S., SURESH, D., SURESH, B.S., KRISHNA, S., MARIRAJ, J., SUREKHA, Y., RAVICHANDRA, P. & RAVIKUMAR, R. 2011. A comparative study of agglutination tests, blood culture and ELISA in the laboratory diagnosis of human brucellosis. *International Journal of Biological and Medical Research*, 2(2), 569-572.

SCHOLZ, H.C., HUBALEK, Z., SEDLACEK, I., VERGNAUD, G., TOMASO, H., AL DAHOUK, S., MELZER, F., KÄMPFER, P., NEUBAUER, H., CLOECKAERT, A., MAQUART, M., ZYGMUNT, M.S., WHATMORE, A.M., FALSEN, E., BAHN, P., GÖLLNER, C., PFEFFER, M., HUBER, B., BUSSE, H.J. & NÖCKLER, K. 2008. *Brucella microti* sp. nov., isolated from the common vole *Microtus arvalis*. *International Journal of Systematic and Evolutionary Microbiology*, 58, 375–382. DOI: 10.1099/ijs.0.65356-0.

SCHOLZ, H.C., HOFER, E., VERGNAUD, G., LE FLECHE, P., WHATMORE, A.M.,AL DAHOUK, S., PFEFFER, M., KRÜGER, M., CLOECKAERT, A. & TOMASO, H. 2009. Isolation of *Brucella microti* from mandibular lymph nodes of red foxes, *Vulpes vulpes*, in lower Austria. *Vector Borne and Zoonotic Diseases*, 9(2), 153-156. DOI: 10.1089/vbz.2008.0036.

SCHURIG, G.G., ROOP, R.M., BAGCHI, T., BOYLE, S., BUHRMAN, D. & SRIRANGANATHAN, N. 1991. Biological properties of RB51; a stable rough strain of *Brucella abortus*. *Veterinary Microbiology*, 28, 171–188. DOI: 10.1016/0378-1135(91)90091-s.

SCHURIG, G.G., SRIRANGANATHAN, N. & CORBEL, M.J. 2002. Brucellosis vaccines: past, present, and future. *Veterinary Microbiology*, 90, 479–496. DOI: 10.1016/s0378-1135(02)00255-9.

SEIFERT, H.S.N. 1996. Brucellosis. Tropical Animal Health. Kluwer Academic Publishers. pp 356- 368.

SELEEM, M.N., BOYLE, S.M. & SRIRANGANATHAN, N. 2010. Brucellosis: A re-emerging zoonosis. *Veterinary Microbiology*, 140, 392–398. DOI: 10.1016/j.vetmic.2009.06.021.

SHARDA, D.C. & LUBANI, M. 1996. A study of brucellosis in childhood. *Clinical Pediatrics*, 25, 492–495. DOI: 10.1177/000992288602501002.

SHEPHERD, A.A., SIMPSON, B.H. & DAVIDSON, R. M. 1980. An economic

evaluation of the New Zealand bovine brucellosis eradication scheme. Paper presented at the Second International Symposium on Veterinary Epidemiology and Economics, Canberra.

SIMPSON, G.J.G., MARCOTTY, T., ROUILLE, E., CHILUNDO, A., LETTESON, J-J. & GODFROID, J. 2018. Immunological response to *Brucella abortus* strain 19 vaccination of cattle in a communal area in South Africa. *Journal of the South African Veterinary Association,* 89, 1527. DOI: 0.4102/jsava.v89i0.1527.

SINGH, B.B., DHAND, N.K. & GILL, J.P.S. 2015. Economic losses occurring due to brucellosis in Indian livestock populations. *Preventive Veterinary Medicine*, 119, 211– 215. DOI: 10.1016/j.prevetmed.2015.03.013.

SINGH, R., BASERA, S.S., TEWARI, K., YADAV, S., JOSHI, S., SINGH, B. & MUKHERJEE, F. 2012. Safety and immunogenicity of *Brucella abortus* strain RB51 vaccine in crossbred cattle calves in India. *Indian Journal of Experimental Biology*, 50, 239–242.

SKALSKY, K., YAHAV, D., BISHARA, J., PITLIK, S., LEIBOVICI, L. & PAUL, M. 2008. Treatment of human brucellosis: systematic review and meta-analysis of randomised controlled trials. *British Medical Journal*, 336(7646), 701–704. DOI: 10.1136/bmj.39497.500903.25.

SMITH, H.L. & KADRI, S.M. 2005. Brucellosis in India: a deceptive infectious disease. *Indian Journal of Medical Research*, 122, 375-384.

SOHN, A.H., PROBERT, W.S., GLASER, C.A., GUPTA, N., BOLLEN, A.W., WONG, J.D., GRACE, E.M. & MCDONALD, W.C. 2003: Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. *Emerging Infectious Diseases*, 9, 485-488. DOI: 10.3201/eid0904.020576.

SOLERA, J. 2010. Update on brucellosis: therapeutic challenges. *International Journal of Antimicrobial Agents*, 36, S18–S20. DOI: 10.1016/j.ijantimicag.2010.06.015.

SOLIS GARCIA DEL POZO, J. & SOLERA, J. 2012. Systematic review and meta-analysis of randomized clinical trials in the treatment of human brucellosis. *PLoS ONE*, 7, e32090. DOI: 10.1371/journal.pone.0032090.

SOLORIO-RIVERA, J.L., SEGURA-CORREA, J.C. & SÁNCHEZ-GIL, L.G. 2007. Seroprevalence of and risk factors for brucellosis of goats in herds of Michoacan, Mexico. *Preventive Veterinary Medicine*, 82, 282-290.

DOI: 10.1016/j.prevetmed.2007.05.024.

SPICKLER, A.R. 2018. Brucellosis: *Brucella abortus*. Retrieved from http://www.cfsph.iastate.edu/DiseaseInfo/ factsheets.php.

STEMSHORN, B.W., NIELSEN, K.H., SAMAGH, B.S., FORBES, L.B. & INGRAM, D.G. 1980. Evaluation of an enzyme-labeled antiglobulin test for anti-*Brucella* immunoglobulin G among three cattle populations. *American Journal for Veterinary Research*, 41, 779-784.

SUÁREZ-ESQUIVEL, M., RUIZ-VILLALOBOS, N., JIMÉNEZ-ROJAS, C., BARQUERO-CALVO, E., CHACÓN-DÍAZ, C., VÍQUEZ-RUIZ, E., ROJAS-CAMPOS, N., BAKER, K.S., OVIEDO-SÁNCHEZ, G., AMUY, E., CHAVES-OLARTE, E., THOMSON, N.R., MORENO, E. & GUZMÁN-VERRI, C. 2017. *Brucella neotomae* infection in humans, Costa Rica. *Emerging Infectious Disease,* 23(6), 997-1000. DOI: 10.3201/eid2306.162018.

SREEVATSAN, S., BOOKOUT, J.B., RINGPIS, F., PERUMAALLA, V.S., FICHT, T.A., ADAMS, L.G., HAGIUS, S.D., ELZER, P.H., BRICKER, B.J., KUMAR, G.K., RAJASEKHAR, M., ISLOOR, S. & BARATHUR, R.R. 2000. A multiplex approach to molecular detection of *Brucella abortus* and/or *Mycobacterium bovis* infection in cattle. *Journal of Clinical Microbiology*, 38(7), 2602-10. DOI:10.1128/JCM.38.7.2602-2610.2000.

SURENDRAN, N., SRIRANGANATHAN, N., LAWLER, H., BOYLE, S.M., HILTBOLD, E.M., HEID, B., ZIMMERMAN, K. & WITONSKY, S.G. 2011. Efficacy of vaccination strategies against intranasal challenge with *Brucella abortus* in BALB/c mice. *Vaccine*, 29(15), 2749-55. DOI: 10.1016/j.vaccine.2011.01.090.

SZULOWSKI, K., IWANIAK, W., WEINER, M. & ZŁOTNICKA, J. 2013. *Brucella suis* Biovar 2 isolations from cattle in Poland. *Annals of Agricultural and Environmental Medicine*, 20(4), 672-675.

TALESKI, V., ZERVA, L., KANTARDJIEV, T., CVETNIC, Z., ERSKI-BILJIC, M., NIKOLOVSKI, B., BOSNJAKOVSKI, J., KATALINIC-JANKOVIC, V., PANTELIADOU, A., STOJKOSKI, S. & KIRANDZISKI, T. 2002. An overview of the epidemiology and epizootology of brucellosis in selected countries of Central and Southeast Europe. *Veterinary Microbiology*, 90(1–4), 147– 155. DOI: 10.1016/s0378-1135(02)00250-x.

TAY, B.Y., AHMAD, N., HASHIM, R., MOHAMED ZAHIDI, A.J., THONG, L.K., KOH, P.X. & MOHD NOOR, A. 2015. Multiple-locus variable-number tandem-repeat analysis (MLVA) genotyping of human *Brucella* isolates in Malaysia. *BMC Infectious. Disease*s, 15, 220. DOI: 10.1186/s12879-015-0958-0.

THRUSFIELD, M. 1995. Veterinary Epidemiology. 2nd Edition, Blackwell Science, Cambridge.

TSOLIS, R.M. 2002. Comparative genome analysis of the alpha -proteobacteria: relationships between plant and animal pathogens and host specificity. *Proceedings of the National Academy of Sciences of the United States of America*, 99 (20), 12503-12505.

TUON, F.F., GONDOLFO, R.B. & CERCHIARI, N. 2017. Human-to-human transmission of *Brucella*—a systematic review. *Tropical Medicine and International Health,* 22, 539–546. DOI: 10.1111/tmi.12856.

TWEDDLE, N.E. & LIVINGSTONE, P. 1994. Bovine tuberculosis control and eradication programs in Australia and New Zealand. *Veterinary Microbiology*, 40(1–2), 23–39. DOI: 10.1016/0378-1135(94)90044-2.

USDA (United States Department of Agriculture). 2003. Animal and Plant Health Inspection Services. Availability of an environmental assessment for licensing of *Brucella abortus* vaccine, Strain RB–51, live culture. Federal Register, 18 Feb 2003, 68, 7761.

VAN METRE, D.C. 1999. Brucellosis induced by RB51 vaccine in a pregnant heifer. *Journal of the American Veterinary Medical Association*, 215(10), 1491-3.

VEMULAPALLI, R., HE, Y., SRIRANGANATHAN, N., BOYLE, S.M. & SCHURIG, G.G. 2002. *Brucella abortus* RB51: enhancing vaccine efficacy and developing multivalent vaccines. *Veterinary Microbiology,* 90, 521–532. DOI: 10.1016/s0378-1135(02)00232-8.

VEMULAPALLI, R., CONTRERAS, A., SANAKKAYALA, N., SRIRANGANATHAN, N., BOYLE, S.M. & SCHURIG, G.G. 2004. Enhanced efficacy of recombinant *Brucella abortus* RB51 vaccines against *B. melitensis* infection in mice. *Veterinary Microbiology*, 102(3-4), 237-45. DOI: 10.1016/j.vetmic.2004.07.001.

VERGER, J.M. 1985. *B. melitensis* infection in cattle. In: *Brucella melitensis*.

PLOMMET M. & VERGER J.M., eds. Martinus Nijhoff Publ., Dordrecht, Netherlands, 197–203.

VERGER, J.M., GRIMONT, F., GRIMONT, P.A.D. & GRAYON, M. 1985. *Brucella*, a monospecific genus as shown by deoxyribonucleic acid hybridization. International *Journal of Systematic and Evolutionary Microbiology*, 35 (3). DOI: 10.1099/00207713-35-3-292.

VILCHEZ, G., ESPINOZA, M., D'ONADIO, G., SAONA, P. & GOTUZZO, E. 2015. Brucellosis in pregnancy: clinical aspects and obstetric outcomes. *International Journal of Infectious Disease*, 38, 95-100. DOI: 10.1016/j.ijid.2015.06.027.

WALKER, R.L. 1999. Brucella. In: D.C. HIRSH & Y.C. ZEE (eds), Veterinary Microbiology, (Blackwell Science Inc.: USA), 196-203.

WANG, Y., WANG, Z., ZHANG, Y., BAI, L., ZHAO, Y., LIU, C., MA, A. & YU, H. 2014. Polymerase chain reaction-based assays for the diagnosis of human brucellosis. *Annals of Clinical Microbiology and Antimicrobials*, 13, 31. DOI: 10.1186/s12941-014-0031-7.

WATTAM, A.R., FOSTER, J.T., MANE, S.P., BECKSTROM-STERNBERG, S.M., BECKSTROM-STERNBERG, J.M., DICKERMAN, A.W., KEIM, P., PEARSON, T., SHUKLA, M., WARD, D.V., WILLIAMS, K.P., SOBRAL, B.W., TSOLIS, R.M., WHATMORE, A.M. & O'CALLAGHAN, D. 2014. Comparative phylogenomics and evolution of the Brucellae reveal a path to virulence. *Journal of Bacteriology*, 196, 920–30. DOI: 10.1128/JB.01091-13.

WEYNANTS, V., GODFROID, J., LIMBOURG, B., SAEGERMAN, C. & LETESSON, J.J. 1995. Specific bovine brucellosis diagnosis based on in vitro antigen-specific gamma interferon production. *Journal of Clinical Microbiology*, 33 (3), 706–712. DOI: 10.1128/JCM.33.3.706-712.1995.

WHATMORE, A.M., SHANKSTER, S.J., PERRETT, L.L., MURPHY, T.J., BREW, S.D., THIRLWALL, R.E., CUTLER, S.J. & MACMILLAN, A.P. 2006. Identification and characterization of variable-number tandem-repeat markers for typing of *Brucella* spp. *Journal of Clinical Microbiology*, 44(6), 1982–1993. DOI: 10.1128/JCM.02039-05.

WHATMORE, A.M. 2009. Current understanding of the genetic diversity of *Brucella*,

an expanding genus of zoonotic pathogens. *Infection, Genetics and Evolution,* 9, 1168–1184. DOI: 10.1016/j.meegid.2009.07.001.

WHATMORE, A.M. & GOPAUL, K.K. 2011. Recent advances in molecular approaches to *Brucella* diagnostics and epidemiology. In: *Brucella*: Molecular Microbiology and Genomics, LÓPEZ-GOÑI I. & O'CALLAGHAN D., eds, Caister Academic Press, Norfolk, UK, 57–88.

WHATMORE, A.M., DAVISON, N., CLOECKAERT, A., AL DAHOUK, S., ZYGMUNT, M.S., BREW, S.D., PERETT, L.L., KOYLASS, M.S., VERGNAUD, G., QUANCE, C., SCHOLZ, H.C., DICK, E.J. JR, HUBBARD, G. & SCHLABRITZ-LOUTSEVITCH, N.E. 2014. *Brucella papionis* sp. nov. isolated from baboons (*Papio* spp.). *International Journal of Systematic and Evolutionary Microbiology*, 64, 4120–4128. DOI: 10.1099/ijs.0.065482-0.

WHATMORE, A.M., KOYLASS, M.S., MUCHOWSKI, J., EDWARDS-SMALLBONE, J., GOPAUL, K.K. & PERRETT, L.L. 2016. Extended multilocus sequence analysis to describe the global population structure of the genus *Brucella*: Phylogeography and relationship to biovars. *Frontiers of Microbiology*, 7, 2049. DOI: 10.3389/fmicb.

WELBURN, S.C., BEANGE, I., DUCROTOY, M.J. & OKELLO, A.L. 2015. The neglected zoonoses--the case for integrated control and advocacy. *Clinical Microbiology and Infection*, 21(5), 433-43. DOI: 10.1016/j.cmi.2015.04.011.

WHO (World Health Organization). 1997. World Health Organisation Fact sheet N173. Geneva, Switzerland: World Health Organization.

WHO (World Health Organization). 2005. Brucellosis in humans and animals. WHO guidance. In: L. HEYMANN (ed), Control of communicable diseases manual: an official report of the American Public Health Association, (World Health Organization/America Public Health Association, Washington DC).

WHO (World Health Organization). 2006. Brucellosis in humans and animals, World Health Organisation in collaboration with the Food and Agricultural Organisation and the World Organisation for Animal Health.

WHO (World Health Organization). 2011. Seven neglected endemic zoonoses-some basic facts. Geneva: World Health Organization. http://www.who.int/zoonoses/neglectedzoonoticdiseases/en/. Accessed 24 August 2019.

YANG, H.X., FENG, J.J., ZHANG, X.Q., HAO, E.R., YAO, X.S., ZHAO, R., PIAO, R.D., CUI, Y.B. & JIANG, H. 2018. A case report of spontaneous abortion caused by *Brucella melitensis* biovar 3. *Infectious Diseases of Poverty*, 7, 31. DOI: 10.1186/s40249-018-0411-x.

YOUNG, E.J. 1991. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. *Review of Infectious Diseases*, 13(3), 359–372. DOI: 10.1093/clinids/13.3.359.

ZHONG, Z., YU, S., WANG, X., DONG, S., XU, J., WANG, Y., CHEN, Z., REN, Z. & PENG, G. 2013. Human brucellosis in the People's Republic of China during 2005–2010. *International Journal of Infectious Diseases*, 17(5). DOI: 10.1016/j.ijid.2012.12.030.

ZINSSTAG, J., SCHELLING, E., ROTH, F., BONFOH, B., DE SAVIGNY, D. & TANNER, M. 2007. Human benefits of animal interventions for zoonosis control. *Emerging Infectious Diseases*, 13(4), 527-31. DOI: 10.3201/eid1304.060381.

Chapter 3: Brucellosis knowledge, attitudes and practices among cattle farmers, meat handlers and medical professionals in Namibia

O. Madzingira[1, 2], C. Byaruhanga [2], F.O. Fasina [3], H. van Heerden [2]

[1] School of Veterinary Medicine, Faculty of Agriculture and Natural Resources, University of Namibia, Namibia

[2] Department of Veterinary Tropical Diseases, Room 2-11, Paraclinical Building University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa.

[3] Emergency Centre for Transboundary Animal Diseases-Food and Agriculture Organisation of the United Nations (ECTAD-FAO), Dar es Salaam

3.1. Abstract

Brucellosis remains a significant zoonosis in many parts of Africa including Namibia. The aim of this study was to assess knowledge, attitudes and practices (KAP) regarding brucellosis among cattle farmers (communal and commercial), meat handlers (abattoir and butchery workers) and medical professionals (nurses and doctors) in selected regions of Namibia between June 2019 and September 2020. A cross-sectional study based on self-administered questionnaires and questionnaire interviews was conducted among cattle farmers (n = 264), meat handlers (n = 143) and medical professionals (n = 124). Overall, 43.50% (231/531) of the respondents were aware of brucellosis, with the highest awareness among medical professionals (73.39%, 91/124) and the least in meat handlers (13.99%, 20/143). Awareness of brucellosis was significantly associated with tertiary education ($p < 0.001$) and the medical profession ($p < 0.001$), although the later engaged in risky practices that could lead to infection. Most medical professionals (98.39%, 122/124) did not consider brucellosis as a differential diagnosis in cases of persistent febrile illness in humans. Most respondents did not consider that they were at risk of contracting brucellosis but were willing to receive information on brucellosis. A proportion of communal (85.60%) and commercial (71.00%) farmers; abattoir workers (44.40%); butchers (53.50%); nurses (55.60%) and medical doctors (28.00%) consumed raw milk, with a high preference for raw milk among adults (31->50 years) ($p = 0.004$), males ($p < 0.001$) and farmers ($p < 0.001$). The study identified practices that may

promote *Brucella* infection and transmission in cattle and humans, such as the purchase of replacement animals without prior health checks; assisting delivery in cows or handling aborted foetuses without protective gear; consumption of raw milk, home-made cheese, cattle testes, and undercooked liver. Therefore, intensified risk communication, including public health education, is recommended, particularly among meat handlers and communal farmers, to promote awareness and discourage risky attitudes and practices that aid *Brucella* infection and transmission.

3.2. Introduction

Brucellosis is a zoonosis of significant socioeconomic, animal, and public health importance (Franc et al., 2018). *Brucella abortus*, *B. melitensis*, *B. suis* and *B. canis* are the species that preferentially infect cattle, sheep and goats, pigs, and dogs respectively, and also cause disease in humans (Moreno, Cloeckaert and Moriyón, 2002). Brucellosis is principally a foodborne or occupation-associated disease in humans that is acquired through the ingestion of infected animal products, especially unpasteurized dairy products or by accidental occupational exposure to infected animal material (Corbel, 1997; Ibironke et al., 2008). Persons that rely on livestock rearing, such as farmers and meat handlers, are at a greater risk of *Brucella* infection (Corbel, 2006), and often, they have limited knowledge of the disease (DAFF, 2017). In animals, infection is self-sustaining and transmitted through direct or indirect contact with or ingestion of infected aborted material (Garin-Bastuji et al., 1998). Due to the similarity of clinical manifestation between brucellosis and other human febrile diseases, misdiagnosis often occurs, leading to inappropriate treatment. Therefore, greater responsibility is placed on individuals to prevent and control the disease (Kiffner et al., 2019).

Brucellosis is an endemic and notifiable disease in both humans and animals in Namibia. Control of the disease is primarily based on compulsory vaccination of heifers of 3 to 8 months of age with *Brucella* S19 or RB51 vaccine in animals older than 8 months; routine serological surveillance and culling of positive reactors (AHR, 2018). In humans, other than the treatment of confirmed cases using a standard six-week treatment protocol of doxycycline and rifampicin, no other measures are specified to actively detect or prevent infection in the population. The continued

detection of cases in the human population in Namibia emphasizes the need to improve disease prevention and control measures in both animal and human populations.

Awareness and knowledge of a disease in a population promote uptake and the correct implementation of disease control measures. Previous studies have linked accurate knowledge of cause, methods of transmission, clinical manifestation, attitudes, and practices with effective control of brucellosis in populations (Howyida et al., 2012; Lindahl et al., 2015). Studies on knowledge, attitude, and practice (KAP) regarding brucellosis among farmers, meat handlers and health professionals can help to identify the extent of knowledge and identify risky practices for *Brucella* infection among human populations. This information is essential for updating current disease prevention and control interventions (Kiffner et al., 2019). Previous studies in South Africa (Cloete et al., 2019), Uganda (Kansiime et al., 2014; Nabirye et al., 2017), Kenya (Obonyo and Gufu, 2015), Jordan (Musallam, Abo-Shehada and Guitian, 2015), Nigeria (Buhari et al., 2015) and Tajikistan (Lindahl et al., 2015) among others, identified varying levels of brucellosis knowledge and awareness among countries, and highlight the need to base brucellosis control measures on identified country-specific knowledge, attitude and practice gaps. Further, in developing countries, such as Namibia, reports indicate that zoonotic diseases such as brucellosis remain a challenge due to insufficient training of health professionals, limited or non-existent collaboration among veterinary and medical practitioners and inadequate health infrastructure (Kunda et al., 2008; Mbugi et al., 2012).

To the best of our knowledge, no studies have been conducted in Namibia to assess knowledge, attitudes and practices relating to brucellosis. Therefore, the current study aimed to assess these among cattle farmers, meat handlers and medical personnel. This should serve as a basis for the development and implementation of more efficient brucellosis control programs that fit the needs and perceptions of the assessed professional groups in Namibia.

3.3. Materials and Methods

3.3.1. Study area

Namibia is in the southwestern part of Africa between -22°58'1.42"S and 18°29'34.80"E and is divided into 14 administrative regions (Figure 3.1). Two distinct farming systems are recognised in the country. In the northern part of the country, farmers raise livestock on communal land close to humans and sometimes wildlife, and grow crops on a subsistence scale. The southern part of the country has predominantly commercial livestock farmers that raise cattle on fenced private land. The northern communal areas are separated from the commercial farms in the southern part by a veterinary cordon fence. Cattle are the major livestock species reared in Namibia, with an estimated population of 2 650 000, 75% of which are in the communal sector (ASB, 2018).

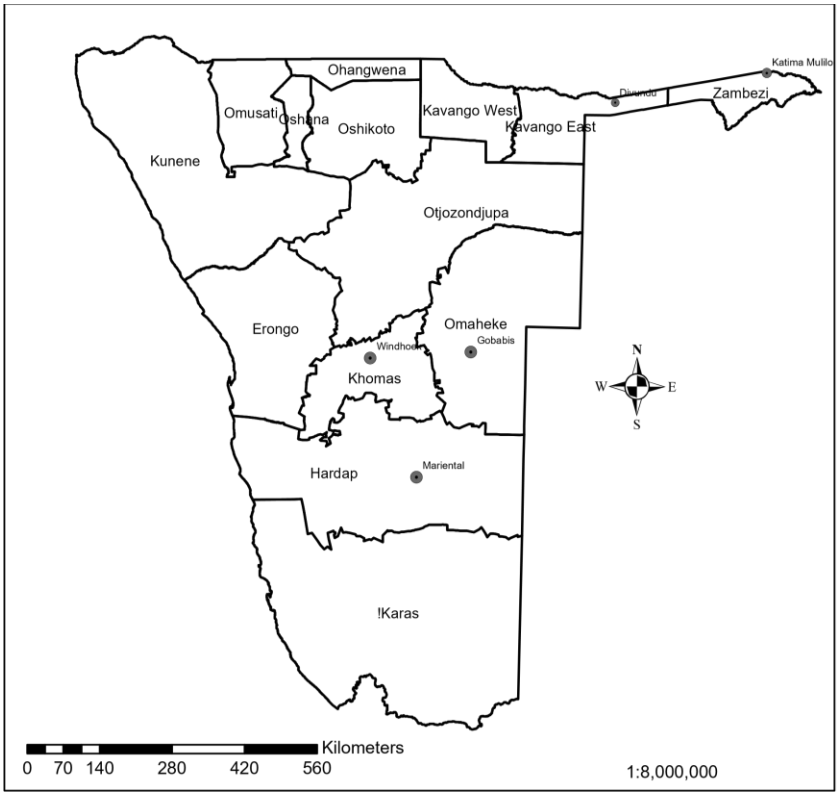


Figure 3.1: The 14 administrative regions of Namibia. The study was carried out in Hardap, Khomas, Omaheke, Kavango East and Zambezi regions.

3.3.2. Study design

Between June 2019 and September 2020, a cross-sectional questionnaire survey was conducted among cattle farmers (communal, commercial), meat handlers (abattoir and butchery workers) and medical workers (nurses and doctors) in both northern communal and southern commercial farming areas, to assess the knowledge, attitudes and practices relating to brucellosis, using pre-tested semi-structured occupation-specific questionnaires. The survey was carried out in five administrative regions of Namibia, namely, Zambezi, Kavango East, Hardap, Khomas and Omaheke (Figure 3.1), which were selected from the 14 regions of the country, using stratified random sampling. The strata were based on production system (communal or commercial) and location (north or south of the country). Abattoir workers were those from the largest and the only beef exporting abattoir in the country, located in the Khomas region, while butchery workers were from butcheries in the urban areas of Divundu (Kavango East), Katima Mulilo (Zambezi), Windhoek (Khomas) and Gobabis (Omaheke). Medical professionals were from private and state medical facilities located in urban areas in all the five study regions: Mariental, Windhoek, Gobabis, Rundu and Katima Mulilo. Respondents of 18 years of age and above that consented to participate in the study were recruited. Participating farmers were identified using systematic random sampling at state veterinary offices, animal auctions and during routine farm inspections. All medical personnel and meat handlers in the selected facilities, who gave consent to participate in the study, were interviewed.

3.3.3. Sample size

The total sample size required for this survey was estimated to be 385 and this was determined using Epitools (https://epitools.ausvet.com.au), assuming an estimated true proportion of 50%, and a precision of 0.05 at 95% level of confidence. However, 531 participants were enrolled in the study to account for any possible clustering effects.

3.3.4. Data collection

Questionnaire interviews (Appendix 1) were carried out by veterinary personnel at state veterinary offices, animal auctions and during routine farm inspection, while some questionnaires were distributed to the farmers through the national farmers' organization. In the communal areas of the Zambezi, Kavango East and the commercial areas of Omaheke, interviews were carried out during farmers' meetings, auctions and at systematically-selected households in the villages. Among butchers and communal farmers, questionnaire interviews were performed by trained veterinary paraprofessionals. Due to the limited numbers of meat handlers in the study areas, all abattoir and butchery employees were targeted. For medical professionals, questionnaires were distributed to medical doctors and nurses at medical facilities in towns located in the five study regions with the permission of the Chief Medical Officer of the area.

The questionnaires for the farmers, butchers and medical professionals were divided into section A to E; A to D and A to B respectively (Appendix 1). Section A was designed to gather the socio-demographic information (age, sex, education level, job title and the number of years on the job of respondents), while Section B gathered information on brucellosis awareness and knowledge including cause, transmission, clinical manifestation and control in both humans and cattle. For medical professionals, information on predisposing practices, and disease cases and management in humans were captured under Section B. Sections C, D and E gathered information on attitudes and predisposing practices for *Brucella* infection.

Verbal or written consent (Appendix 2) was sought prior to the interviews depending on the respondent's literacy levels. Data were collected independently from each participant and coded to ensure anonymity and confidentiality at all stages of the study. To ensure the understanding of questions, interviewers translated the questions to the native language as necessary.

3.3.5. Data analyses

Data from close-ended responses were presented as frequencies and percentages per question, while open-ended responses were categorized into themes and summarized. The chi-square or Fisher's exact tests were used to determine the significance of differences between proportions of respondents for each variable: between communal and commercial farmers, between nurses and doctors, and between abattoirs and butchery workers.

For multi-variable analysis, six variables that were common to all participant categories (farmers, medical workers, meat handlers), and considered to be of importance in exposure to brucellosis were considered. This Multiple Correspondence Analysis (MCA) was performed to determine patterns in the dataset, whereby different categories of each column and row variables, and the relations between them, were depicted as 'clouds' of points in a multidimensional Euclidean space. The variables were age of respondent (18-30, 31-50, >50 years), gender, education level (no formal education, primary, secondary, tertiary), profession (nurse, doctor, commercial farmer, communal farmer, butcher worker, abattoir worker), number of years at work (<2 yeas, 2 to 5 years, >5 years), awareness about brucellosis (yes, no), and consumption of raw milk and its products (yes, no). Interpretation of the results was made on basis of the relative distribution and position of points across the dimensions. The number of MCA dimensions to retain was determined using the eigenvalue criteria. The coordinates (*coord*), quality of representation (*cos2*) and contribution (*contrib*, %) of each variable to the dimensions on the factor map were determined.

The MCA analysis was followed by cluster analysis of individual participants and categories with the object scores of each dimension, using Hierarchical Clustering on Principal Components (HCPC). Cross-tabulations were performed between cluster variables in the MCA using the chi-square tests for proportions. Data analysis was performed using R Console version 4.0.3 (R Core Team, 2020) at 5% level of significance.

3.3.6. Ethical approval

Authorization to carry out the study at the abattoir was obtained from the abattoir operator. The study protocol was approved by the Ministry of Health and Social Services (Ref: 17/3/3 OM) (Appendix 10) and the Research Ethics Committees of the University of Pretoria (REC056-20 and HUM026/0620) (Appendix 6 and Appendix 7).

3.4. Results

3.4.1. Sociodemographic characteristics of respondents

A total of 531 respondents, that included 264 cattle farmers (communal = 195, commercial = 69), 143 meat handlers (abattoir employees = 72, butchers = 71) and 124 medical professionals (nurses = 99, medical doctors = 25) from the Hardap (n = 57), Khomas (n = 119), Omaheke (n = 100), Kavango East (n = 87) and Zambezi (n = 168) regions of Namibia participated in the survey. There were more male (68.74%, n = 365) than female (31.26%, n = 166) respondents. The demographic features of the study groups are shown in Table 3.1.

3.4.2. Knowledge of brucellosis

Overall awareness of brucellosis among participants was 43.5% (231/531), with the highest frequency recorded among medical professionals (73.4%, 91/124), followed by cattle farmers (45.5%, 120/264) and the least was among meat handlers (14.0%, 20/143).

The primary sources of information on brucellosis among respondents were the workplace or professional colleagues (46.3%, 107/231), training institutions (school, college or university) (26.0%, 60/231), veterinary officials (14.3%, 33/231), media (10.4%, 24/231), training workshops/profession meetings (3.9%, 9/231), literature (2.2%, 5/231) and the Ministry of Health and Social Services (0.4%, n = 1/231).

Table 3.1: Socio-demographic features of the survey respondents (n = 531) from five regions of Namibia.

Variable	Category	Communal farmers (n=195) %	Commercial farmers (n=69) %	Abattoir employees (n=72) %	Butchers (n=71) %	Nurses (n=99) %	Medical doctors (n=25) %
Age	18-30	9.7	7.3	38.9	32.4	49.5	24.0
	31-50	53.9	55.1	58.3	62.0	42.4	60.0
	>50	36.4	37.7	2.8	5.6	8.1	16.0
Gender	Male	73.9	91.3	72.2	78.9	33.3	68.0
	Female	26.2	8.7	27.8	21.1	66.7	32.0
Education level	No formal education	12.3	2.9	0.0	16.9	0.0	0.0
	Primary	14.9	14.5	6.9	25.4	0.0	0.0
	Secondary	36.4	46.4	80.6	46.5	0.0	0.0
	Tertiary	36.4	36.2	12.5	11.3	100.0	100.0
Region	Hardap	0.0	46.4	0.0	0.0	22.2	12.0
	Omaheke	21.5	53.6	0.0	11.3	13.1	0.0
	Kavango East	13.3	0.0	0.0	53.5	19.2	16.0
	Zambezi	65.1	0.0	0.0	16.9	24.2	20.0
	Khomas	0.0	0.0	100.0	18.3	21.2	52.0

The survey was conducted to determine the knowledge, attitudes and practices regarding brucellosis

3.4.2.1. Communal and commercial farmers

Knowledge of brucellosis was higher among commercial than communal farmers ($p < 0.05$) (Table 3.2). Less than 30.0% (range: 7.7 - 27.7%) of communal farmers had knowledge about the animal species affected by brucellosis; its transmission; zoonotic nature; symptoms or clinical signs; prevention and management approaches in both humans and animals (Table 3.2). In contrast, more than half of the commercial farmers (29.0 - 79.7%) had knowledge on most aspects of

brucellosis, although only small proportions had knowledge of the clinical manifestation of the disease in cattle (29.0%) and prevention measures in humans (33.3%) (Table 3.2).

Both communal and commercial farmers identified undercooked meat and raw milk (7.2%, n = 14 and 20.3%, n = 14, respectively) and direct contact with infected animal material (2.6%, n = 5 and 27.5%, n = 19, respectively) as the major sources of *Brucella* infection in humans. Other identified sources of human infection were accidental inoculation with *Brucella v*accine (n = 1) and blood transfer (n = 1). Overall, the symptoms of human brucellosis reported by the farmers were fever (9.1%), headache (1.9%), back pain (3.4%), profuse sweating at night (1.9%), fatigue (1.5%), joint pain (3.0%) and body aches (3.0%) and swollen joints (1.5%). Farmers associated abortions (15.9%), swollen joints (4.5%) and swollen testes (4.2%) with bovine brucellosis.

Table 3.2: Knowledge of brucellosis among communal and commercial farmers in selected regions in Namibia.

Variable	Communal farmers (n = 195)		Commercial farmers (n = 69)		
	Frequency		Frequency		
	N	%	N	%	*p* value
Species affected	54	27.7	55	79.7	< 0.001*
Mode of transmission to humans	20	10.3	35	50.7	< 0.001*
Mode of transmission to cattle	21	10.8	37	53.6	< 0.001*
Symptoms in humans	15	7.7	30	43.5	< 0.001*
Clinical signs in cattle	27	13.9	20	29.0	0.005*
Prevention in humans	15	7.7	23	33.3	< 0.001*
Prevention in cattle	42	21.5	52	75.4	< 0.001*

* Statistically significant at 5% level of significance. The participating farmers were selected from Gobabis, Hardap, Kavango, Khomas and Zambezi regions in Namibia. N = number of respondents. Proportions were compared using the chi-square test of association.

3.4.2.2. Meat handlers

Few abattoir and butchery workers (0.0-16.7%) had knowledge of brucellosis (species affected, transmission, symptoms, and prevention) (Table 3.3). Apart from knowledge about prevention of brucellosis in humans and cattle, whose frequency was higher among abattoir workers (15.3% and 16.7%, respectively) than butchery workers (2.8% and 4.2% respectively) ($p < 0.05$) (Table 3.3), other aspects of brucellosis showed no significant difference between the two categories of meat handlers ($p > 0.05$) (Table 3.3). Majority of meat handlers were unable to mention any zoonotic disease (88.1%) or cause of abortion in cattle (91.6%).

Table 3.3: Knowledge of brucellosis among meat handlers (abattoir and butchery workers) (n = 143) in selected regions of Namibia.

Variable	Abattoir workers (n = 72) Frequency		Butchery workers (n = 71) Frequency		
	N	%	N	%	*p* value
Species affected	10	13.9	4	5.6	0.16
Mode of transmission to humans	4	5.6	3	4.2	0.72
Mode of transmission to cattle	4	5.6	3	4.2	1.0
Symptoms in humans	5	6.9	0	0	0.06
Clinical signs in cattle	2	2.8	2	2.8	1.00
Prevention in humans	11	15.3	2	2.8	0.02*
Prevention in cattle	12	16.7	3	4.2	0.03*

* Statistically significant at 5% level of significance. The participating meat handlers were selected from one abattoir and 35 butchers in four regions (Gobabis, Khomas, Kavango East and Zambezi) in Namibia. N=number of respondents. Proportions were compared using the Fisher's exact test of association.

3.4.2.3. Medical professionals

All medical doctors (25/25) and most of the nurses (68.7%, 68/99) had knowledge of the mode of transmission of brucellosis to humans, and the difference was significant ($p < 0.001$). A higher proportion of medical doctors (92.0%) than nurses (71.7%)

gave the correct advice regarding the prevention of *Brucella* infection in humans (p = 0.04).

Although most medical doctors (96.0%) and nurses (82.8%) had encountered cases of persistent fever in patients, only 1.6% of the healthcare respondents (two medical doctors and none of the nurses) listed brucellosis as part of the differential diagnosis. Other differential diagnoses listed were malaria, tuberculosis, urinary tract infection, respiratory tract infection, meningitis, HIV/AIDS, influenza (H1N1), COVID-19 and typhoid fever in descending order (data not shown). Also included in the list of differentials were deep abscess, rheumatic fever, autoimmune disease, drug fever, cat-scratch disease, toxoplasmosis, tick-bite fever, leptospirosis, hay fever, dental-related diseases, and Epstein-Barr viral infection (data not shown).

3.4.3. Attitudes towards brucellosis

3.4.3.1. Farmers

More commercial (53.6%) than communal (30.3%) farmers considered raw milk to be as healthy as pasteurized milk sold in shops ($p < 0.05$) (Table 3.4). However, the majority of communal (85.6%) and commercial (68.1%) farmers boiled raw milk before consuming it ($p < 0.05$). Although most communal (96.9%) and commercial farmers (89.8%) considered abortions as a profoundly serious occurrence in cattle herds, high proportions of communal (75.4%) and commercial (82.6%) farmers treated diseases in their herds without consulting a veterinary official. Furthermore, majority of communal (96.4%) and commercial (82.6%) did not consider themselves at risk of brucellosis. Overall, 92.8% of farmers from both farming sectors will need more information on brucellosis (Table 3.4).

Table 3.4: Frequency of responses from communal and commercial farmers (n = 264) showing their attitudes towards brucellosis and possible sources of infection in Namibia.

Variable	Communal farmers (n = 195) Frequency		Commercial farmers (n = 69) Frequency		
	N	%	N	%	*p* value
Raw milk or home-made cheese is as healthy as similar products sold in retail shops					
Yes	59	30.3	37	53.6	< 0.001*
No	134	68.7	28	40.6	
Don't know	2	1.0	4	5.8	
It is necessary to boil raw milk before drinking it					
Yes	167	85.6	47	68.1	0.001*
No	26	13.3	17	24.6	
Don't know	2	1.0	5	7.2	
Seriousness of cattle abortions					
Very serious	125	64.1	53	76.8	0.001*
Serious	64	32.8	9	13.0	
Not important	6	3.1	7	10.1	
Handling of diseased cattle					
Treat myself	159	81.5	57	82.6	< 0.001*
Seek veterinary help	68	34.9	19	27.5	
Slaughter for meat	4	22.1	2	2.9	
Do nothing	0	0	2	2.9	
Risk of *Brucella* infection					
Yes	60	30.8	10	14.5	0.006*
No	120	61.5	47	68.1	
Don't know	15	7.7	12	17.4	
Need for brucellosis information					
Yes	188	96.4	57	82.6	< 0.001*
No	7	3.6	12	17.4	

* Statistically significant at 5% level of significance. The survey was conducted in five of the 14 regions in Namibia. N=number of participants. Proportions compared using the chi-square or Fisher's exact tests of association.

3.4.3.2. Meat handlers

About 45.5% (n = 65) of meat handlers regarded raw cow's milk and homemade cheese as having the same health status as pasteurized products sold in the shops. More abattoir than butchery workers considered raw milk to be as safe as pasteurized milk (61.1% vs 29.6%) and handling of aborted foetus or stillbirth materials (43.1% vs 19.7%) as unsafe with regards to *Brucella* infection ($p < 0.001$) (Table 3.5). Overall, a high proportion of respondents were averse to handling (67.1%) or drinking of cattle blood (90.2%) and to the opening of a cow's uterus without protective gear (76.9%). Although more butchery workers (32.4%) than abattoir workers (18.1%) ($p = 0.004$) mentioned that it was safe to handle blood from slaughtered cattle with unprotected hands, the perception of risk of *Brucella* infection at work was higher among butchery (38.0%) than abattoir workers (27.8%) ($p = 0.037$) (Table 3.5).

Table 3.5: Responses of abattoir and butchery workers (n = 143) showing their attitudes towards sources of *Brucella* infection at home and the work place in selected regions of Namibia.

Variable	Abattoir workers (n=72) Frequency		Butchery workers (n=71) Frequency		
	N	%	N	%	*p* value
Raw milk or home-made cheese is as healthy as similar products sold in retail shops					
Yes	44	61.1	21	29.6	< 0.001*
No	28	38.9	50	70.4	
Handling aborted or stillborn calves with no protection can result in *Brucella* infection					
Yes	31	43.1	14	19.7	< 0.001*
No	19	26.4	12	16.9	
Don't know	22	30.6	45	63.4	
It is safe to handle blood from slaughtered cattle with unprotected hands					
Yes	13	18.1	23	32.4	0.004*
No	57	79.2	39	54.9	
Don't know	2	2.8	9	12.7	
It is safe to drink blood from slaughtered cattle.					
Yes	13	18.1	2	2.8	0.003*
No	57	79.2	63	88.7	
Don't know	2	2.8	6	8.5	
Risk of *Brucella* infection at work					
Yes	20	27.8	27	38.0	0.037*
No	44	61.1	43	60.6	
Don't know	8	11.1	1	1.4	

***** Statistically significant at 5% level of significance. The survey was conducted in three of the 14 regions in Namibia. N=number of respondents. Proportions were compared using the chi-square or Fisher's exact tests of association.

3.4.3.3. Medical professionals

Although most of the nurses (64.6%) and doctors (84.0%) did not think they were at risk of *Brucella* infection, a high proportion (94.9% and 88.0% respectively) indicated

that they needed more information on the disease. The difference in opinion between the two groups was not significant ($p > 0.05$) (Table 3.6).

Table 3.6: Responses of medical workers (n = 124) in five regions of Namibia regarding their attitudes towards brucellosis.

Variable	Nurses (n=99) Frequency		Medical doctors (n=25) Frequency		
	N	%	N	%	*p* value
Risk of *Brucella* infection					0.171
Yes	34	34.3	4	16	
No	64	64.6	21	84	
Don't know	1	1.0	0	0	
Need for more information on brucellosis					0.206
Yes	94	94.9	22	88	
No	5	5.1	3	12	

***** Statistically significant at 5% level of significance. Data was collected using semi-squared interviews.

3.4.4. Practices that promote *Brucella* infection and transmission

3.4.4.1. Farmers

Significantly higher proportions of communal farmers than commercial farmers engaged in practices that promote *Brucella* infection, including consumption of raw milk from cattle, sheep or goats, assisting cows to give birth, frequent mixing of different herds, and use of communal bulls ($p < 0.05$). Furthermore, more communal farmers grazed cattle with other animal species, and purchased replacement stock from other farmers and auctions ($p > 0.05$) (Table 3.7). Milking of cows by hand was a common practice among communal (94.4%, n = 184) and commercial (76.8%, n = 53) farmers (data not shown).

Table 3.7: Practices that promote *Brucella* infection in humans among communal and commercial farmers in Namibia.

Variable	Communal farmers (n=195) Frequency		Commercial farmers (n=69) Frequency		
	N	%	N	%	*p* value
Raw milk consumption					
Yes	167	85.6	49	71.0	0.007*
No	28	14.4	20	29.0	
Handling of aborted fetuses with bare hands					
Yes	62	31.8	22	31.9	0.99
No	133	68.2	47	68.1	
Assisting cows to deliver					
Yes	127	65.1	34	49.3	0.035*
No	68	34.9	35	50.7	
Raising cattle with other animal species (e.g. goats or sheep)					
Yes	72	36.9	58	84.1	<0.001*
No	123	63.1	11	15.9	
Purchase of replacement cattle from outside (e.g. other farmers and auctions)					
Yes	166	85.1	51	73.9	0.036*
No	29	15.9	18	26.1	
Veterinary health checks on replacement animals					
Yes	149	84.2	39	56.5	0.003*
No	28	15.8	14	20.3	
Frequency of contact with other herds					
Always	147	75.4	0	0	<0.001*
Sometimes	42	21.5	3	4.3	
Rarely	1	0.5	8	11.6	
Don't mix at all	5	2.6	58	84.1	
Breeding method					
Own bull	161	82.6	69	100.0	<0.001*
Communal bull	34	17.4	0	0	

***** Statistically significant at 5% level of significance. Survey conducted in five of the 14 regions in Namibia. Proportions compared using the chi-square and Fisher's exact tests of association.

The rate of vaccination against brucellosis in communal cattle herds was estimated at 19.5% (n = 38) and 87.0% (n = 60) in commercial herds. Cattle were vaccinated using *Brucella* S19 or RB51 vaccines. No *Brucella* positive cattle were reported by commercial farmers. However, one communal farmer reported a case of a seropositive cow that remained in the herd. Aborted foetuses and membranes were left on pastures (29.9%, 79/264), fed to dogs (28.8%, 76/264), buried (29.9%, 79/264) or burned (6.4%, 27/264).

3.4.4.2. Meat handlers

Practices that could promote *Brucella* infection among abattoir and butchery workers were identified as splashing of blood into the eyes, drinking raw milk, eating undercooked or raw meat/viscera, assisting cows to deliver with bare hands, and eating home-made cheese. Both categories of meat handlers were at similar risk of infection ($p > 0.05$) (Table 3.8). However, no abattoir worker handled meat or animals with bruised or injured hands (Table 3.8). In the event of a hand injury, the workers were either withdrawn from working with meat until healed (31.9%, n = 23), or the injured part was covered with waterproof dressing (45.8%, n = 33) before proceeding with their routine work. On the other hand, 7.0% (n = 5) of the butchers stated that they handled meat using unprotected hands following injuries (Table 3.8). All abattoir workers were provided with full protective gear, while 38.0% (n = 27) of the butchers did not use any protective gear.

Table 3.8: Practices that may promote *Brucella* infection as mentioned by abattoir and butchery workers in Namibia.

Variable	Abattoir workers (n=72) Frequency		Butchery workers (n=71) Frequency		
	N	%	N	%	*p* value
Consumption of raw milk or home-made cheese					
Yes	32	44.4	38	53.5	0.32
No	40	55.6	33	46.5	
Handle cattle fetuses during carcass dressing					
Yes	8	11.1	13	18.3	0.25
No	64	88.9	58	81.7	
Frequency of blood splash into the eyes					
Always	3	4.2	3	4.2	0.89
Sometimes	17	23.6	16	22.5	
Rarely	17	23.6	13	18.3	
Never	35	48.6	39	54.9	
Eat cattle testicles, uteri, undercooked or raw meat					
Yes	35	48.6	26	36.6	0.18
No	37	51.4	45	63.4	
Handle meat with bare hands following injury					
Yes	0	0	5	7.0	0.03*
No	72	100	66	93.0	

* Statistically significant at 5% level of significance. The survey was conducted in five of the 14 regions of Namibia, using semi-structured questionnaires. N=number of respondents. Proportions were compared using chi-square and Fisher's exact test.

3.4.4.3. Medical professionals

More nurses (55.6%, 55/99) than medical doctors (28.0%, 7/25) (p = 0.014) consumed raw milk and/or other dairy products such as home-made cheese and

yoghurt (Table 3.9). Although only one medical doctor and six nurses assisted cows to deliver without hand protection, four medical doctors and 34 nurses indicated that they were at risk of *Brucella* infection.

Table 3.9: Practices that were identified as likely to expose medical practitioners to *Brucella* infection in five regions in Namibia.

Variable	Nurses (n=99)		Medical doctors (n=25)		
	Frequency		Frequency		
	N	%	N	%	*p* value
Consumption of raw milk or dairy products					
Yes	55	55.6	7	28	0.014*
No	44	44.4	18	72	
Assistance to cows during delivery with no protection					
Yes	6	6.1	1	4	1.0
No	93	93.9	24	96	

* Statistically significant at 5% level of significance. A semi-structured questionnaire survey was conducted in five of the 14 regions in Namibia. All nurses and medical doctors in 10 private and public health facilities in the five regions (Hardap, Gobabis, Khomas, Kavango East and Zambezi) were interviewed.

All communal cattle farmers (n = 195) and meat handlers (n = 143) had not seen a case of human brucellosis in their lifetime, while four commercial cattle farmers, three nurses and two medical doctors had seen a total of 13 cases of the disease. The source of infection in the observed cases were reported as raw milk consumption and contact with live or dead cattle. The clinical signs observed were fever, poor appetite, back pain, joint pain, general body aches, headaches, and night sweats. Other symptoms included body weakness, vomiting, abdominal pain, diarrhoea, weight loss, skin rashes, swollen liver, and lymph nodes. Treatment in medical facilities was by administration of doxycycline and rifampicin for six weeks, which resulted in complete recovery of all patients.

3.4.5. Mode of receiving information

Respondents received information on brucellosis through veterinary officials (13.6%, n = 72), radio programs (13.2%, n = 70), training workshops (9.2%, n = 49), pamphlets (6.4%, n = 34), email (3.0%, n = 16), posted mail (1.5%, n = 8), telephonically (0.8%, n = 4), short message service (sms) (0.4%, n = 2), farmers' magazine (0.2%, n = 1) and newspapers (0.2%, n = 1).

3.4.6. Multivariable analysis

Multiple correspondence analysis of seven variables (age, gender, education, occupation, years at work, awareness, consumption of raw milk and products) showed that dimensions 1 and 2 were sufficient to retain 29% of the total variation (inertia) contained in the data. The variables that contributed most to the two dimensions, and therefore the most important in explaining variations in the dataset were: age 18 to 30 years (16.5%), nurse (20.2%), work for less than 2 years (12.7%) and awareness (yes) about brucellosis (15.0%), while other variables, including age 31-50 (0.9%), raw milk consumption (yes) (2.8%) and male (2.4%) contributed the least to the dimensions.

The distance of the variable profiles from the origin of the factor map was evaluated. Categories such as medical doctor, nurse and commercial farmers that lie far from the origin of the map reflect variation (special pattern) from the average (Figure 3.2). Thus, all medical doctors (100.0%, n = 25), most nurses (67.0%, 66/99) and commercial farmers (84.1%, 58/69) were aware of brucellosis, and in both categories of medical profession, all individuals had tertiary education (Figure 3.2).

3.4.6.1. Awareness of brucellosis

Age was not significantly associated with awareness of brucellosis, with more than 50.0% of each age category found to be unaware (p = 0.8) (Figure 3.2), and there was little or no association between gender (female, male) (each <50.0%) with awareness about the disease (p = 0.04). Evaluation of education levels showed a good association between awareness and tertiary education (p < 0.001), but no association was observed for primary, secondary or lack of formal education. Awareness was associated with medical practice; most nurses (67.0%, 66/99) and

all medical doctors (n = 25) were aware of the disease (p < 0.001). On the other hand, awareness was less with the other occupations, that is, butcheries (8.5%, 6/71), abattoirs (19.4%, 14/72) and communal farmers (31.8%, 62/195) (Figure 3.2). Overall, there was no significant association between awareness of brucellosis and consumption of raw milk (p = 0.62) (Figure 3.2). Therefore, although a large proportion of commercial farmers drank raw milk and/or milk products (71.0%, 49/69), this category was aware about brucellosis (84.0%, 58/69) (Figure 3.2). For the number of years at work, individuals who had worked for more than 5 years were more aware about brucellosis than those who had worked for less than 2 years or 2 to 5 years (Figure 3.2).

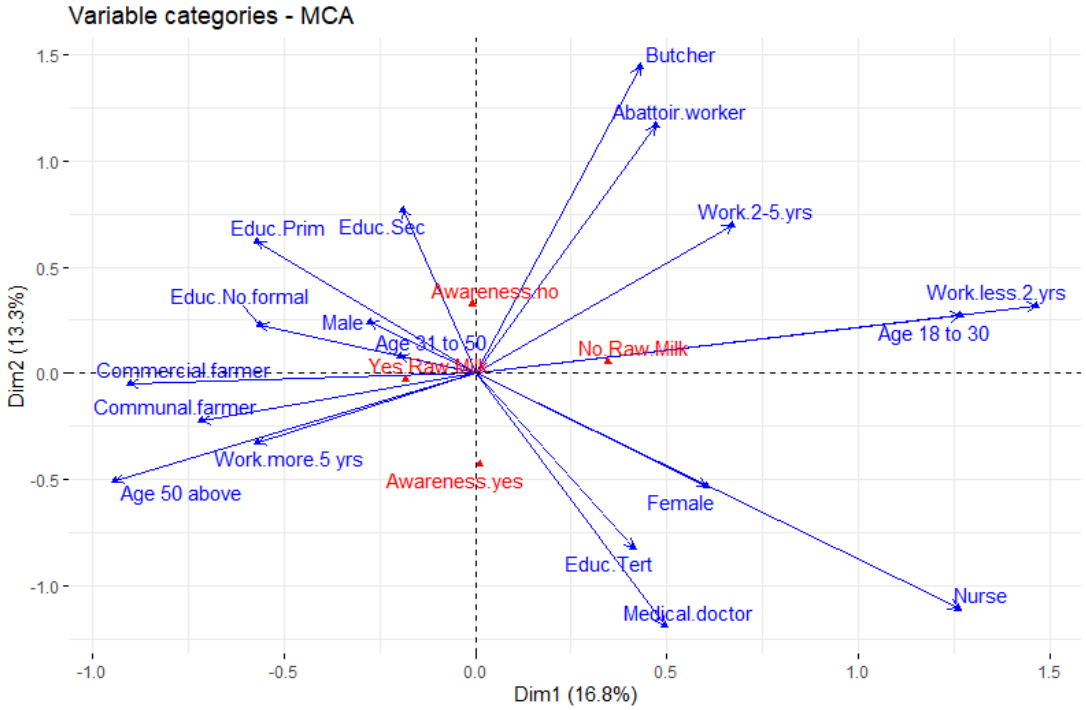


Figure 3.2: A symmetric biplot of the first two axes of a multiple correspondence analysis (MCA) showing the association between potential risk factors related to human brucellosis in Namibia. The variables included were education level, occupation, gender, age, duration at work, consumption of raw milk/milk products, and awareness of brucellosis. A total of 531 participants (195 communal farmers, 69 commercial farmers, 71 butchery workers, 72 abattoir workers, 99 nurses and 25 medical doctors) were interviewed from five regions, namely Hardap, Khomas, Kavango East, Omaheke and Zambezi.

3.4.6.2. Consumption of raw milk and milk products

The consumption of raw milk and milk products was associated more with older age categories, >50 years (72.2%, 83/115) and 31-50 years (68.2%, 195/286), than with the younger category, 18-30 years, (53.8%, 70/130) (p = 0.004) (Figure 3.2). Analysis of gender with respect to consumption of raw milk showed that the practice was more common among males (69.9%, 255/365) than females (56.0%, 93/166) (p = 0.002) (Figure 3.2). A similar pattern of association was observed between consumption of raw milk and the four education categories (p = 0.23), that is, 76.9% for no formal education, 66.7% for primary, 67.8% for secondary and 61.6% for tertiary (Figure 3.2). Relatively high proportions of communal farmers (85.6%, 167/195) and commercial farmers (71.0%, 49/69) consumed raw milk (p < 0.001), but the practice was not common among abattoir workers, butchery workers and medical personnel (Figure 3.2). Assessment of the participants' longevity in their occupations showed that longer duration (>5 years) (71.3%, 238/334) was linked to consumption of raw milk and its products, but little or no association of the practice was observed with shorter duration in occupation (<2 years, 2-5 years) (Figure 3.2). Interestingly, about a half (43.7%, 232/531) of the people with more years in occupation were farmers.

3.4.6.3. Cluster analysis

Cluster analysis showed that demographic characteristics and potential risk factors for brucellosis led to partitioning of the participants into three clusters (Figure 3.3 and Table 3.10). The variables that most significantly influenced the clustering of participants were occupation (p < 0.001) and education (p < 0.001), based on the chi-square test (Table 3.10). The first cluster comprised mainly the communal and commercial farmers, work for more than five years, and age above 50 (Figure 3.2 and Figure 3.3). The second cluster comprised butchery and abattoir workers, while medical personnel (nurses and doctors) constituted the third cluster (Figure 3.2 and Figure 3.3).

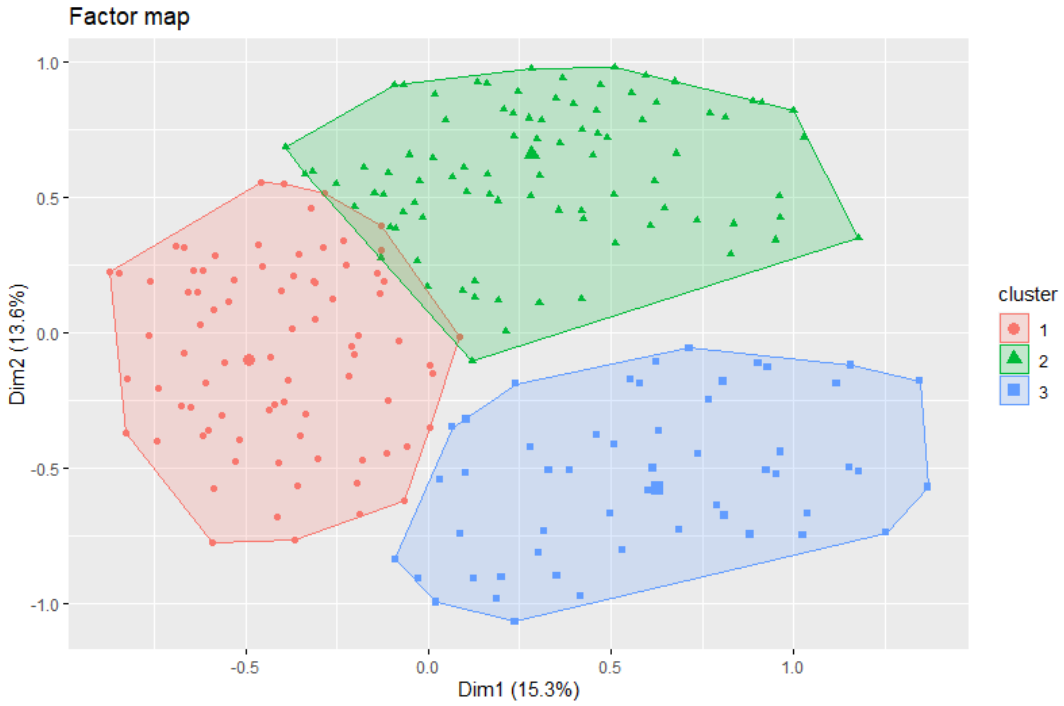


Figure 3.3: Factor map showing clustering of individual participants by demographic (education, occupation, age, years at work, gender) and risk factors (awareness, raw milk consumption) for brucellosis. Cluster 1: communal and commercial farmers, work for more than five years, and age above 50; Cluster 2: butchery and abattoir workers; Cluster 3: medical personnel (nurses and doctors). Data was collected during questionnaire interviews of 531 participants in five regions of Namibia.

Table 3.10: Variables that significantly influenced clustering of participants based on knowledge, attitudes and practices concerning brucellosis in Namibia.

Variable	p-value	Degrees of freedom
Occupation	1.46E-196	10
Education	1.27E-48	6
Years at work	4.94E-43	4
Age	1.75E-30	4
Awareness about brucellosis	6.16E-25	2
Consumption of raw milk and milk products	2.38E-15	2
Gender	2.71E-15	2

The 531 participants were drawn from five regions in Namibia. Cluster analysis was determined using chi-square test.

3.5. Discussion

Cases of brucellosis have been confirmed in humans (Magwedere et al., 2011) and domestic ruminants (Madzingira et al., 2014, 2015, 2016, 2020) in Namibia. Hence, a 'One Health' approach was used to assess knowledge, attitudes and practices in farmers, meat handlers and medical professionals with a view to guide brucellosis control programmes and public health interventions in the country.

The current study found that 43.5% of the respondents were aware of brucellosis, which is lower than awareness levels reported in Nigeria (92.9%) (Buhari et al., 2015), Uganda (99.3%) (Kansiime et al., 2015) and Egypt (83.0%) (Safaan and Mohsen, 2016). Previous studies have shown that brucellosis awareness and prevalence are positively correlated (Govindaraj et al., 2016). Therefore, the low awareness recorded in the current study may be related to the low prevalence of brucellosis in Namibia (Madzingira et al., 2014; 2015; 2016; 2020) and has the potential to negatively impact compliance with brucellosis control measures and surveillance for the disease in the country (Ruano and Aguayo, 2017). Multivariable analysis confirmed that brucellosis awareness was particularly low among communal cattle farmers (31.8%), abattoir workers (19.4%) and butchers (8.5%), the majority of whom had limited education (63.6%, 87.5% and 88.7% respectively), when compared to participants with tertiary education (nurses and doctors). Studies in other countries have reported similar findings (Lindahl et al., 2015; Ruano and Aguayo, 2017), highlighting the need to focus public health education on these groups. Brucellosis awareness increased with the number of years spent in an occupation, showing that experiential learning is an important component of brucellosis knowledge acquisition (Govindaraj et al, 2016). Previous studies have implicated low literacy rates, lack of health education programs, limited training on animal handling and rearing procedures, limited extension services, absence of health facilities and the remote location of participants as causes of low brucellosis awareness (Munyeme et al., 2010).

In this study, communal cattle farmer awareness and knowledge of brucellosis was significantly lower than that recorded in commercial cattle farmers. Awareness among communal farmers in this study (31.8%) was about half the awareness level

(60.0%) of communal farmers in South Africa (Cloete et al., 2019). Previous studies have implicated limited access to animal health resources and services (FAO, 2001; Grace et al., 2017) as causes of low animal disease knowledge among communal farmers. In Namibia, this was confirmed by Haakuria et al. (2020) and may be the reason, in part, why most farmers treated animals without consulting veterinary personnel. Although a high proportion of commercial farmers (84.1%) were aware of brucellosis, they lacked in-depth knowledge of the disease, particularly, on the clinical manifestation of the disease in cattle and prevention of the disease in humans. This may explain why a large proportion of these farmers underappreciated the risk of infection with brucellosis and consumed raw milk. In contrast to a report by Cloete et al. (2019), the current study showed a minor role for state veterinary services (14.3%) in promoting brucellosis awareness, with the workplace (46.3%) and training institutions (26.0%) playing a major role.

Brucellosis awareness and knowledge was low among meat handlers. As expected, awareness was higher at the highly regulated export abattoir than at the less regulated urban butcheries. The frequency of brucellosis awareness among butchers (8.5%) was comparable to findings of a similar study in India (11.0%) (Singh and Jindal, 2017), while awareness levels in abattoirs workers (19.4%) were lower than the level (76.0%) reported in Tanzania (Luwumba et al., 2019). The low level of brucellosis knowledge that was recorded in meat handlers (2.8-16.7%), contrasts the higher levels recorded in Ethiopia (44.2%) and Tanzania (76.0%) (Tsegay et al., 2017) (Luwumba et al., 2019). Despite the apparent lack of brucellosis knowledge, it was encouraging that ≤ 7.0% of meat handlers handled carcasses without protection or with bruised or injured hands. Results of this study show that most abattoir and butchery operators provided protective wear to their workers but did not provide adequate training on zoonotic disease to meat handlers. Therefore, an intensified risk communication strategy involving abattoir and butchery operators is recommended to reduce potential exposure to *Brucella* infection among meat handlers.

Knowledge on various epidemiological aspects of brucellosis including the mode of transmission and prevention in humans was more frequent amongst medical

professionals (more than two-thirds), than other participant categories, as was also observed in Uganda (Nabirye et al., 2017). However, brucellosis was rarely considered during diagnosis of febrile illnesses among patients that visited medical facilities, with only two medical doctors considering it and none of the nurse respondents. The multiplicity of diseases that present with similar non-specific clinical symptoms in Namibia such as malaria and typhoid, may have played a role. Therefore, as has also been observed in Tanzania (Zhang et al., 2016), active surveillance for human brucellosis at medical facilities around the country may be low. With a third (33.3%) of the nurses lacking brucellosis knowledge, misdiagnosis of brucellosis within the population and the subsequent development of the severe chronic disease in patients due to delayed or no treatment cannot be ruled out (Kunda et al., 2007; Kunda, Kazwala and Mfinanga, 2008; Dean et al., 2012; Nabirye et al., 2017). Such a situation is likely to be more prevalent in remote areas of the country where in the absence of physicians, nurses make the initial diagnosis. With a contribution of only 0.43% to awareness among the respondents, the Ministry of Health and Social Services needs to play an increased role in the dissemination of zoonotic disease information to the Namibian population.

Most of the farmers (91.3%), meat handlers (67.8%) and medical professionals (68.5%) did not perceive that they were at risk of *Brucella* infection and were therefore likely to engage in practices that pose a risk for *Brucella* infection such as drinking raw milk, assisting cow parturition and disposal of aborted foetuses without wearing protective gloves (Ruano and Aguayo, 2017). Multivariable analysis revealed no significant association between awareness of brucellosis and consumption of raw milk. The absence of an association between participants' knowledge and practices has also been observed by other studies (Mangesho et al., 2021) and explains the following findings from this study: 1) approximately half of the farmers and meat handlers regarded raw milk and pasteurized milk from shops as of same health status; 2) respondents' education did not affect their attitude towards consuming raw milk and milk products; 3) and individuals with more work experience were more likely to consume raw milk and milk products. Although the consumption of raw milk was linked to farmers, the majority of communal (85.6%) and commercial farmers (68.1%) boiled raw milk before consuming it, which may be the reason why

most farmers did not think that they were at risk of *Brucella* infection. Further positive attitudes were recorded in most of the farmers and meat handlers regarding the handling of blood with bare hands, drinking cattle blood and the opening of the uterus, which reduce possible exposure to infection. It was also encouraging that most farmers, meat handlers and medical professionals requested education on brucellosis, an observation that was also made among participants in Uganda (Kansiime et al., 2015). The identified positive attitudes can be used as a basis for improving knowledge of the disease among the respondents through health education.

The likelihood of consumption of raw milk and milk products increased with age of respondents, and was more common among males than females, and among communal farmers than commercial farmers. Therefore, old age did not seem to result in a change of eating habits. The fact that more males than females consumed raw milk may reflect the closeness and dependency of male livelihoods on animals in the Namibian society, as has been reported elsewhere (Grahn, 2013; Cleaveland et al., 2017).

In the communal areas, the study identified regular contact among cattle herds (75.4%); low brucellosis vaccination rates (19.5%) and the use of communal bulls (17.4%) as practices that may promote *Brucella* infection and transmission between and within herds. It is difficult to control brucellosis in a pastoral system where regular contact between herds occurs (Sammartino, Gil and Elzer, 2005), but the vaccination of cattle can be promoted to reduce abortions and disease incidence. The low vaccination rate identified in the communal sector (19.5%) compared to the commercial sector (87%) may be due to low awareness of the disease among farmers; a lack of resources to implement the vaccination program (FAO, 2001) or limited enforcement of the compulsory vaccination of heifers 3-8 months of age using the S19 vaccine by government officials (Madzingira et al., 2020). On commercial farms, the rearing of cattle in a mixed farming system with domestic or wild animal species (85.5%) and the sourcing of replacement cattle from outside the farm by most farmers (82.6%) are practices that can promote the introduction and persistence of infection due to several reservoirs. On a positive note, both communal

(76.4%) and commercial (56.5%) farmers performed health assessments on replacement cattle before purchase, which though not specific, can contribute to brucellosis prevention on farms. The practices implemented by most commercial farmers including the absence of contact between different cattle herds (84.1%), vaccination of cattle (87.0%), seeking veterinary help in case of suspected brucellosis (60.9%), and the use of own breeding bulls promote the prevention and control of the disease on farms.

The high level of awareness and knowledge of brucellosis that was observed in medical professionals did not translate into good practices to prevent *Brucella* infection, as has been observed by other studies (Arif et al., 2017). For example, some medical doctors consumed partially roasted meat sold by street vendors, and came in contact with cattle fluids with no protection and consumed raw milk (28.0%). Furthermore, some nurse respondents consumed home-made cheese and yoghurt, undercooked meat, raw meat such as biltong; handled cattle blood or consumed meat of doubtful health status and more than half consumed raw milk (55%). Similarly, a large proportion of commercial farmers (71%) drank raw milk, despite being aware that brucellosis can be transmitted to humans through raw dairy products. Results of this study agree with previous reports that high levels of brucellosis awareness and knowledge do not necessarily translate into appropriate behaviours and practices, because perception of risk is influenced by several factors including life experiences and culture (Sjoberg, 2000). Therefore, a shift in behaviour and cultural practices may be necessary to reduce the risk of exposure of humans to brucellosis as earlier reported (Njenga et al., 2020).

Based on the relatively low levels of brucellosis awareness and knowledge determined in this study, public health education and awareness campaigns are recommended as the main strategy for risk mitigation. Such campaigns, led by public health and veterinary officials, and following a One Health approach, should focus on communal farmers, abattoir workers and butchers. Awareness regarding the boiling of raw milk before consumption, the wearing of protective gear such as gloves when assisting cow delivery or handling aborted animal tissues, and the vaccination of cattle against brucellosis should be emphasised. Disease awareness material should

be designed and delivered to the public based on the data generated in the current study. Furthermore, medical professionals should be regularly sensitised to consider brucellosis as a differential diagnosis for febrile conditions. It is also recommended that the Ministry of Health and Social Services develop a strategy for surveillance and control of brucellosis in the country. Future studies to determine the burden of *Brucella* infection in occupationally-exposed groups are recommended.

The limitation of this study was the low response rate among medical doctors compared to other groups. This was due to the small number of doctors in the regions of study, which was also compounded by their busy schedules. However, because the study covered four regions, the results can be taken as representative of the situation in the country. Furthermore, one cattle abattoir was included in the study because it was the only high throughput abattoir that was operational in the country at the time.

3.6. Conclusion

The study identified brucellosis knowledge, attitude and practice deficiencies that may predispose humans to serious public health effects and reduce cattle production. Awareness and knowledge of the disease were particularly low among communal farmers, abattoir, and butchery workers. Practices that are a risk for human infection with *Brucella* spp. including the consumption of raw milk and associated dairy products, consumption of undercooked meat, splashing of blood into the eyes during slaughter and assisting cows during delivery were identified among the study groups.

3.7. References

AHR (Animal Health Regulations). 2018. Government Notice 358 of 2018. Animal Health Act 1 of 2011. Retrieved from: https://laws.parliament.na/cms_documents/animal-health-act-1-of-2011---regulations-2018-358-d37ff9f7a0.pdf.

ARIF, S., THOMSON, P.C., HERNANDEZ-JOVER, M., MCGILL, D.M., WARRIACH, H.M. & HELLER, J. 2017. Knowledge, attitudes and practices (KAP) relating to brucellosis in smallholder dairy farmers in two provinces in Pakistan. *PloS One*, *12*(3), e0173365. DOI: 10.1371/journal.pone.0173365.

ASB (Agricultural Statistics Bulletin). 2018. Ministry of Agriculture, Water and Land Reform. https://mawf.gov.na/web/mawf/annual-reports.

BUHARI, H.U., SAIDU, S.N.A., MOHAMMED, G. & RAJI, M.A. 2015. Knowledge, attitude and practices of pastoralists on bovine brucellosis in the north senatorial district of Kaduna state, Nigeria. *Journal of Animal Health and Production*, 3**,** 28–34. DOI: 10.14737/journal.jahp/2015/3.2.28.34.

CLEAVELAND, S., SHARP, J., ABELA-RIDDER, B., ALLAN, K.J., BUZA, J., CRUMP, J.A., CLEAVELAND, S., SHARP, J., ABELA-RIDDER, B., ALLAN, K. J., BUZA, J., CRUMP, J. A., DAVIS, A., DEL RIO VILAS, V. J., DE GLANVILLE, W. A., KAZWALA, R. R., KIBONA, T., LANKESTER, F. J., LUGELO, A., MMBAGA, B. T., RUBACH, M. P., SWAI, E. S., WALDMAN, L., HAYDON, D. T., HAMPSON, K. & HALLIDAY, J. 2017. One Health contributions towards more effective and equitable approaches to health in low- and middle-income countries. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *372*(1725), 20160168. DOI: 10.1098/rstb.2016.0168.

CLOETE, A., GERSTENBERG, C., MAYET, N. & TEMPIA, S. 2019. Brucellosis knowledge, attitudes and practices of a South African communal cattle keeper group. *Onderstepoort Journal of Veterinary Research* 86(1), 10. DOI: 10.4102/ ojvr.v86i1.1671.

CORBEL, M.J. 1997. Brucellosis. An overview. *Emerging Infectious Diseases*, 3(2), 213–221. DOI: 10.3201/eid0302.970219.

CORBEL, M.J. 2006. Brucellosis in humans and animals. Food and Agriculture

Organization of the United Nations, World Health Organization and World Organisation for Animal Health. Brucellosis in humans and animals. World Health Organization. https://apps.who.int/iris/handle/10665/43597.

DAFF (Department of Agriculture, Forestry & Fisheries). 2017. Discussion paper on the review of bovine brucellosis control in South Africa, Directorate Animal Health, DAFF, Pretoria.

DEAN, A.S., CRUMP, L., GRETER, H., SCHELLING, E. & ZINSSTAG, J. 2012. Global burden of human brucellosis: A systematic review of disease frequency. *PLoS Neglected Tropical Diseases*, 6(10), e1865. DOI: 10.1371/journal.pntd.0001865.

FAO (Food and Agricultural Organization of the United Nations). 2001. Farming Systems and Poverty. Improving farmers' livelihoods in a changing world. http://www.fao.org/3/Y1860E/y1860e00.htm. Accessed on January 16, 2020.

FRANC, K.A., KRECEK, R.C., HÄSLER, B.N. & ARENAS-GAMBOA, A.M. 2018. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health,* 18, 125. DOI: 10.1186/s12889-017-5016-y.

GARIN-BASTUJI, B., BLASCO, J.M., GRAYON, M. & VERGER, J.M. 1998. *Brucella melitensis* infection in sheep: present and future. *Veterinary Research,* 29, 255.

GOVINDARAJ, G., NAGALINGAM, M., NETHRAYINI, K.R., SHALINI, R., RAJESWARI, S., BAMBAL, R.G., LIPI, S. & RAHMAN, H. 2016. Assessment of brucellosis knowledge, attitude and practice among veterinarians in India. *Journal of Experimental Biology and Agricultural Sciences*, 4, S83–S94. DOI: 10.18006/2016.4(SPL-3-ADPCIAD).S83.S94.

GRACE, D., LINDAHL, J., WANYOIKE, F., BETT, B., RANDOLPH, T. & RICH, K. 2017. Poor livestock keepers: ecosystem poverty-health interactions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 19, 372 (1725). DOI: 10.1098/rstb.2016.0166.

GRAHN C. 2013. Brucellosis in small ruminants - an Investigation of knowledge, attitude and practices in peri-urban farming around the region of Dushanbe, Tajikistan. Uppsala, 1652-8697, pp. 1-22. https://stud.epsilon.slu.se/5325/7/grahn_c_130227.pdf.

HAAKURIA, V.M., PYATT, A.Z. & MANSBRIDGE, S.C. 2020. Exploration of veterinary service supply to rural farmers in Namibia: a one health perspective. *PAMJ - One Health*, 2(17). DOI: 10.11604/pamj-oh.2020.2.17.24658.

HOWYIDA, S.A., LAMIAA, T.A. & KAMEL, A.Z. 2012. Awareness of personnel in direct contact with animals regarding brucellosis. *Journal of American Science*, 8, 790-796.

IBIRONKE, A.A., MCCRINDLE, C.M., FASINA, F.O. & GODFROID, J. 2008. Evaluation of problems and possible solutions linked to the surveillance and control of bovine brucellosis in sub-Saharan Africa, with special emphasis on Nigeria. *Veterinaria Italiana*, 44, 549– 556.

KUNDA, J., KAZWALA, R. & MFINANGA, G.S. 2008. Knowledge of causes, clinical features and diagnosis of common zoonoses among medical practitioners in Tanzania. *BMC Infectious Diseases*, *8*, 162. DOI: 10.1186/1471-2334-8-162**.**

KANSIIME, C., MUGISHA, A., MAKUMBI, F., MUGISHA, S., RWEGO, I.B., SEMPA, J., KIWANUKA, S.N., ASIIMWE, B.B. & RUTEBEMBERWA, E. 2014. Knowledge and perceptions of brucellosis in the pastoral communities adjacent to Lake Mburo National Park, Uganda. *BMC Public Health*, 14**,** 242. DOI: 10.1186/1471-2458-14-242.

KIFFNER, C., LATZER, M., VISE, R., BENSON, H., HAMMON, E. & KIOKO, J. 2019*.* Comparative knowledge, attitudes, and practices regarding anthrax, brucellosis, and rabies in three districts of northern Tanzania. *BMC Public Health,* 19, 1625. DOI: 10.1186/s12889-019-7900-0.

KUNDA, J., FITZPATRICK, J., KAZWALA, R., FRENCH, N.P., SHIRIMA, G., MACMILLAN, A., KAMBARAGE, D., BRONSVOORT, M. & CLEAVELAND, S. 2007. Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC Public Health*, *7*, 315. DOI: 10.1186/1471-2458-7-315.

LINDAHL, E., SATTOROV, N., BOQVIST, S. & MAGNUSSON, U. 2015. A study of knowledge, attitudes and practices relating to brucellosis among small-scale dairy farmers in an urban and peri-urban area of Tajikistan. *PLoS One*, 10, e0117318. DOI: 10.1371/journal.pone.0117318.

LUWUMBA, D., KUSILUKA, L. & SHIRIMA, G. 2019. Occupational hazards

associated with human brucellosis in abattoir settings: A case study of Dodoma abattoir in Tanzania. *Journal of Veterinary Medicine and Animal Health*, 11(3), 73-80. DOI: 10.5897/JVMAH2019.0752.

MADZINGIRA, O. & MCCRINDLE, C. 2014. Prevalence of *Brucella* antibodies in sheep and springbok (*Antidorcas marsupialis*) reared together in the Karas region, Namibia. *Bulletin of Animal Health and Production in Africa*, 62, 299-306.

MADZINGIRA, O. & MCCRINDLE, C. 2015. Retrospective analysis of the prevalence of *Brucella* antibodies in sheep in the Karas Region of Namibia. *Tropical Animal Health and Production* 47, 1117. DOI: 10.1007/s11250-015-0838-z.

MADZINGIRA, O. & MCCRINDLE, C. 2016. A questionnaire survey of risk factors of brucellosis on mixed sheep and springbok (*Antidorcas marsupialis*) farms. *International Science and Technical Journal of Namibia,* 8, 43-49.

MADZINGIRA, O., FASINA, F.O., KANDIWA, E., MUSILIKA-SHILONGO, A., CHITATE, F. & VAN HEERDEN, H. 2020. A retrospective sero-epidemiological survey of bovine brucellosis on commercial and communal farming systems in Namibia from 2004 to 2018. *Tropical Animal Health and Production, 52*(6), 3099-3107. DOI: 10.1007/s11250-020-02332-4.

MAGWEDERE, K., BISHI, A., TJIPURA-ZAIRE, G., EBERLE, G., HEMBERGER, Y., HOFFMAN, L.C. & DZIVA, F. 2011. Brucellae through the food chain: the role of sheep, goats and springbok (*Antidorcus marsupialis*) as sources of human infection in Namibia. *Journal of the South African Veterinary Association*, 82, 205-212. DOI: 10.4102/jsava.v82i4.75.

MANGESHO, P.M., CAUDELL, M.A., MWAKAPEJE, E., OLENESSELLE, M., KIMANI, T., DORADO-GARCÍA, A., KABALI, E. & FASINA, F.O. 2021. Knowing is not enough: A mixed-methods study of antimicrobial resistance knowledge, attitudes, and practices among Maasai pastoralists. *Frontiers in Veterinary Science*, 8, 645851. DOI: 10.3389/fvets.2021.645851.

MBUGI, E.V., KAYUNZE, K.A., KATALE, B.Z., KENDALL, S., GOOD, L., KIBIK, G.S., KEYYU, J.D., GODFREY-FAUSSETT, P., VAN HELDEN, P. & MATEE, M.I. 2012. 'One Health' infectious diseases surveillance in Tanzania: are we

all on board the same flight? *Onderstepoort Journal of Veterinary Research*, 19, 79(2), 500. DOI: 10.4102/ojvr.v79i2.500.

MORENO, E., CLOECKAERT, A. & MORIYÓN, I. 2002. *Brucella* evolution and taxonomy. *Veterinary Microbiology*, 90, 209-227. DOI: 10.1016/s0378-1135(02)00210-9.

MUNYEME, M., MUMA, B., MUNANGANDU, H., KANKYA, C., SKJERVE, E. & TRYLAND, M. 2010. Cattle owners' awareness of bovine tuberculosis in high and low prevalence settings of the wildlife-livestock interface areas in Zambia. *BMC Veterinary Research*, 6, 21. DOI: 10.1186/1746-6148-6-21.

MUSALLAM, I.I., ABO-SHEHADA, M.N. & GUITIAN, J. 2015. Knowledge, attitudes and practices (KAP) associated to brucellosis in animals of the livestock owners of Jordan. *American Journal of Tropical Medicine and Hygiene*, 93 (6), 1148-1155. DOI: 10.4269/ajtmh.15-0294.

NABIRYE, H.M., ERUME, J., NASINYAMA, G.W., KUNGU, J.M., NAKAVUMA, J., ONGENG, D. & OWINY, D.O. 2017. Brucellosis: Community, medical and veterinary workers' knowledge, attitudes, and practices in Northern Uganda. *International Journal of One Health*, 3, 12-18. DOI: 10.14202/IJOH.2017.12-18.

NJENGA, M.K., OGOLLA, E., THUMBI, S.M., NGERE, I., OMULO, S., MUTURI, M., MARWANGA, D., BITEK, A., BETT, B., WIDDOWSON, M.A., MUNYUA, P. & OSORO, E.M. 2020. Comparison of knowledge, attitude, and practices of animal and human brucellosis between nomadic pastoralists and non-pastoralists in Kenya. *BMC Public Health*, *20*(1), 269. DOI: 10.1186/s12889-020-8362-0.

OBONYO, M. & GUFU, W.B. 2015. Knowledge, attitude and practices towards brucellosis among pastoral community in Kenya, 2013. *International Journal of Innovative Research and Development* 4(10), 375–384.

R CORE TEAM. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www. R-project.org/.

RUANO, M.P. & AGUAYO, M.D.Z. 2017. Study of knowledge about bovine brucellosis among people involved in the cattle value chain in the province of Manabí, Ecuador. *Revue scientifique et technique (International Office of Epizootics)*, 36 (3), 1-22. DOI: 10.20506/rst.36.3.2725.

SAFAAN, N.A. & MOHSEN, M.M. 2016. Farmers' awareness regarding brucellosis as a neglected emerging infectious diseases in rural areas. *International Journal of Novel Research in Healthcare and Nursing* 3, 35–51.

SAMMARTINO, L.E., GIL, A. & ELZER, P. 2005. Capacity building for surveillance and control of zoonotic diseases. Rome, Italy: Food and Agriculture Organization of the United Nations. Available from: http:// www.fao.org/docrep/009/a0083e/a0083e00.HTM.

SINGH, A. & JINDAL, P. 2017. Awareness and practices among butchers of unorganised slaughterhouses of Punjab regarding zoonotic diseases. *Research in Environmental and Life Sciences*, 10(10), 824–827.

SJOBERG, L. 2000. Factors in risk perception. *Risk Analysis*, 20(1), 1-12. DOI: 10.1111/0272-4332.00001.

TSEGAY, A., TULI, G., KASSA, T. & KEBEDE, N. 2017. Seroprevalence and risk factors of brucellosis in abattoir workers at Debre Zeit and Modjo export abattoir, Central Ethiopia. *BMC infectious diseases*, *17*(1), 101. DOI: 10.1186/s12879-017-2208-0.

ZHANG, N., HUANG, D., WU, W., LIU, J., LIANG, F., ZHOU, B. & GUAN, P. 2018. Animal brucellosis control or eradication programs worldwide: A systematic review of experiences and lessons learned. *Preventive Veterinary Medicine*, 160, 105–15. DOI: 10.1016/j.prevetmed.2018.10.002.

Chapter 4: Seroprevalence of brucellosis among suspect human cases presenting at health facilities in Namibia from 2012 to 2017.

Oscar Madzingira[1, 2], Folorunso Oludayo Fasina[2, 3], Chester Kalinda [4], Henriette van Heerden [2]

[1] School of Veterinary Medicine, Faculty of Agriculture and Natural Resources, University of Namibia, P. Bag 13301, Windhoek, Namibia
[2] Department of Veterinary Tropical Diseases, Room 2-11, Paraclinical Building University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
[3] Emergency Centre for Transboundary Animal Diseases-Food and Agriculture Organisation of the United Nations (ECTAD-FAO), Dar es Salaam
[4] University of KwaZulu-Natal, Howard College Campus, Department of Public Health, College of Health Sciences, School of Nursing and Public Health, Durban 4001, South Africa

4.1. Abstract

Human brucellosis is an underreported and re-emerging zoonotic disease of public health importance worldwide. Occurrence of the disease in the human population is linked to its presence in the animal population. Secondary data comprising human brucellosis serological testing results (2012-2017) were analyzed to estimate the prevalence, determine distribution of cases and to identify plausible risk factors for the disease. Serological testing was carried out at an accredited state laboratory, the Namibia Institute of Pathology laboratory, using a commercial rapid agglutination test. Seroprevalence was 11.64% (113/971, 95% CI: 9.77-13.81%). Annual prevalence of infection among the tested individuals doubled from 2012 (14.07%) to 2017 (28.97%), despite an obvious decline in the number of presumptive cases. Most of the positive cases (56.32%, n = 98) originated from private medical facilities and were clustered in the 30-40 year age group. Brucellosis prevalence increased with age up to the 30-40-year age range and thereafter declined. There were more female reactors (64%) than males (36%) ($z = -5.24$, $p < 0.01$). Disease incidence was reported in all 14 regions of Namibia. Regional incidence of brucellosis in

humans ranged from 0.49 per 100 000 (Oshikoto) to 44.27 per 100 000 Hardap). National incidence rate was determined to be 6.96 per 100 000 population. Among the regions, the risk of infection was not significant (RR: 0.997, 95% CI: 0.971 - 1.02) except for the Erongo region (RR: 0.398, 95% CI: 0.164 - 0.962). Both age category (RR: 0.980, 95% CI: 0.907 - 1.05) and gender (RR: 0.800, 95% CI: 0.598 - 1.069) were not risk factors for the disease. This study revealed the prevalence of brucellosis in the Namibian population, with incident cases reported in all regions of the country. A One Health approach towards the prevention and control of the disease is recommended.

4.2. Introduction

Brucellosis is a zoonosis of global importance that is caused by *Brucella* spp. *Brucella abortus* and *B. melitensis* are the main pathogens responsible for disease in humans and production losses in cattle, sheep, and goats. Human infection is acquired by direct contact with infected animals or indirectly through the consumption, contact with (Kuplulu and Sarimehmetoglu, 2004) or inhalation of infected material from animal reservoirs (Chukwu, 1987). Unpasteurized milk, home-made cheese, and ice cream, as well as meat, present a risk for human infection (Fensterbank, 1986; FAO/WHO, 1986; Chukwu, 1987; Leclerc et al., 2002; Doganay and Aygen, 2003; Kuplulu and Sarimehmetoglu, 2004; Godfroid et al, 2005; Corbel, 2006). Brucellosis is an occupational hazard for professionals who are in regular contact with animals and animal products, such as veterinarians, butchers, and livestock farmers (Chukwu, 1987; Boschiroli et al., 2001; EC, 2001; Godfroid, 2002; Moyer and Holocomb, 2005).

Human infection manifests as an acute, subacute, or chronic infection with non-specific symptoms that are consistently accompanied by an intermittent fever. Other symptoms associated with brucellosis include headache, night sweats, back pain, chronic fatigue, polyarthritis, pneumonia, meningitis, weight loss, abortions, and prostration (EC, 2001; Godfroid, 2002; Pappas et al., 2005; Al Dahouk, 2007). Chronic brucellosis has a negative impact on the social and economic facets of affected patients, as patients spend many days away from work and treatment is prolonged and expensive (WHO, 2006; Zinsstag et al., 2007).

Brucellosis is often overlooked by public health authorities in developing countries (WHO, 2006; FSAI, 2009; Hotez and Gurwith, 2011; Godfroid et al., 2011) due to inadequate monitoring, insufficient human resource and laboratory capacity for diagnosis or surveillance (Fournier et al., 2015) as well as the presence of other acute febrile illnesses, such as malaria, typhoid, paratyphoid, and influenza that confound clinical diagnosis of the disease (Renukaradhya et al., 2002). As a result, the infection is re-emerging worldwide (Godfroid, 2002; Pappas, 2010), with global estimates of over 500 000 (Pappas, 2010) to 12 500 000 cases per year (Godfroid et al., 2013; Berger, 2016). Global burden of the disease is estimated at <0.03 to >160 per 100 000 population (Küstner, 1985; Taleski et al., 2002; Pappas et al., 2006; Seleem et al., 2010).

Diagnosis of human brucellosis is primarily based on serological assays, although isolation is the gold standard test. Screening and confirmation of presumptive cases can be done using rapid agglutination tests, the standard agglutination test (SAT), the Rose Bengal test (RBT) (Lucero et al., 2007; Zeytinoglu et al., 2018), Coombs Test, Complement Fixation Test or the enzyme-linked immunosorbent assay (ELISA) (WHO, 2006). In Namibia, a commercial rapid slide agglutination test (Fortress Diagnostics Limited, UK, www.fortressdiagnostics.com), is used to screen patients for *Brucella* infection. This test kit is also used in many African countries to confirm clinical cases of brucellosis (de Glanville et al., 2017). Although PCR is an effective assay for detecting *Brucella* infection, it has not yet been validated for use on human specimens in Namibia.

Reports of human brucellosis in Namibia are limited. In the only report identified in the literature, an outbreak of human brucellosis was linked to the consumption of goat derived raw milk and home-made cheese (Magwedere et al., 2011). However, serological evidence of the disease in cattle, sheep and goats abounds from previous studies (Madzingira and McCrindle, 2014; Madzingira and Sezuni, 2017) and annual reports of the Ministry of Agriculture, Water and Land Reform (Directorate of Veterinary Services). As in other African countries, the epidemiology of brucellosis and its burden on the human population are poorly described (Foster

et al., 2018). Therefore, the aim of this study was to estimate the prevalence of infection and to identify plausible risk factors for the disease based on serological testing data collected from 2012 to 2017. It is anticipated that the data obtained will provide evidence based empirical information to assist public health and veterinary authorities in formulating and refining strategies for preventing and controlling the disease in the country.

4.3. Material and Methods

4.3.1. Study area

Namibia is located at -22°58'1.42"S and 18°29'34.80"E in the southwestern part of Africa and covers a total land area of 824 292 km² (Info-Namibia, 2019). It is divided into 14 administrative regions and has an estimated total population of 2.5 million inhabitants. The northern and north-eastern parts of the country are densely populated communal areas where the human population lives close to domestic ruminants and grazing is communal. The southern part of the country has predominantly large commercial livestock farms that are sparsely stocked and populated. Daytime temperatures vary from around 20˚C at the coast to 50˚C in the desert. Namibia has two main seasons, the hot summer or rainy season (October to March) and the cool winter or dry season (April to September).

4.3.2. Study design

This study was a cross-sectional sero-epidemiological study on presumptive clinical cases of human brucellosis (2012-2017) that were screened for brucellosis at the Namibia Institute of Pathology between 2012 and 2017. The data were used to describe the demographic characteristics (age, sex), prevalence and distribution of confirmed cases at regional and country level and to identify plausible risk factors. For the purposes of this study, a presumptive or suspected case of brucellosis was defined as a patient who visited a certified private or state physician, and after clinical evaluation, was suspected to have brucellosis-like symptoms and whose blood sample was submitted to the laboratory for brucellosis screening. A positive or confirmed case of brucellosis was considered as a presumptive or suspected case that showed a positive titre on the agglutination test.

4.3.3. Data collection

Human brucellosis testing results from around the country (2012 to 2017) were retrieved from the Namibia Institute of Pathology (NIP) database with prior authorization of the Ministry of Health and Social Services. The data were filtered to retain only the cases that met the case definition for presumptive and confirmed cases of brucellosis. For each case, the date of sampling, patient identification code, location of health facility, age, sex, and the outcome of the serological test were recorded. Patient exposure history and clinical symptoms were missing from the data, and thus, were not part of this study.

4.3.4. Serological tests

Serological diagnosis for brucellosis was carried out at the NIP using a commercial rapid slide agglutination test kit (Fortress Diagnostics Limited, United Kingdom) as the principal assay, following the manufacturer's procedure. The test used standardized and stained *B. abortus* (FEBBAB05) and *B. melitensis* (FEBBME05) somatic 'O' antigens (febrile antigens) to detect antibodies against *Brucella* spp. in human serum. Briefly described, reagents and sera were brought to room temperature and the antigen (*B. abortus* and *B. melitensis*) was thoroughly reconstituted before use. A drop of 50 µl of test serum was mixed with a drop of 50 µl of *B. abortus* antigen on a test card circle using a disposable stirrer. The serum and antigen mixture were then placed on a mechanical rotator at 100 revolutions per minute (rpm) for two minutes. Results were read under a bright artificial light by checking for the presence or absence of agglutination (clumping) within a minute of removal from the rotator. Results were validated by comparing the outcome to the positive and negative controls that were supplied with the kit and run for each test batch. As part of quality assurance program of the laboratory, the quality of test antigens was routinely verified using known positive and negative sera.

Samples that tested positive on the rapid slide agglutination test were confirmed and titres determined using the semi-quantitative agglutination test by the same test kit manufacturer (Fortress Diagnostics Limited, United Kingdom). In this test, different volumes (80, 40, 20, 10 and 5 µl) of undiluted patient serum were placed on five separate test circles (1 to 5 respectively) using a micropipette and 50 µl of *Brucella* spp. antigen were added to each test circle. Test serum and antigen were mixed

using a disposable stirrer and placed on a mechanical rotator at 100 rpm for two minutes. Observation for agglutination was done as described for the qualitative test. The positive titres were determined as the highest dilution that showed agglutination, while negative results were those that did not show any agglutination. Agglutination in the first, second, third, fourth or fifth well was considered as representing a 1:20, 1:40, 1:80, 1:160 or 1:320 titre, respectively. Positive and negative controls were prepared for each run of tests and used to validate the performance of the test.

4.3.5. Data analyses

Brucellosis testing results were stored in Microsoft Excel® spreadsheet version 2007 (Microsoft Corporation, USA) and exported to STATA version 15 for data analysis. Demographic data were analyzed using descriptive statistics. The *z*-test was used to test the significance of differences between the proportion of reactor males and females. The cumulative incidence of reactors was used to estimate prevalence, temporal, and spatial distributions over the study period. Spatial distribution of positive cases was mapped using GIS in ArcView. Incidence was estimated per region and for the country as the proportion of seropositive cases in the population over the six-year study period and determined for the 100 000th person. *P* values < 0.05 were taken as statistically significant. A log binomial regression model was used to determine the effect of region on relative risk (RR) of infection. Relative risk for age and gender were entered into a multivariable log binomial model.

4.3.6. Ethical approval

The study protocol was approved by the Ministry of Health and Social Services (Namibia) (Appendix 10) and the Research Ethics Committees of the University of Pretoria (REC056-20 and HUM026/0620) (Appendices 6 and 7).

4.4. Results

4.4.1. Patient demographics

A total of 971 presumptive cases of brucellosis were tested for anti-*Brucella* antibodies over the six-year study period. The number of patients with suspected *Brucella* infection declined from 199 (20.5%) cases in 2012 to 107 (11.0%) cases in 2017.The median age of the patients was 36 years (interquartile range (IQR) 25 - 49 years). Most of the participants were aged between 30 and 40 years (26.78%, n = 260), followed by those who aged between 41-50 years (18.02%, n = 175), 20 - 29 years (16.58%, n = 161), 51-60 years (15.65%, n = 152), 13 – 19 years (8.55%, n = 83), 61 – 84 years (6.18%, n = 60), 6 – 12 years (5.56%, n = 54), and 0 – 5 years (2.68%, n = 26). Most patients were females (59.32%, n = 576, $z = 5.7$, $p < 0.001$).

4.4.2. Overall prevalence of positive cases

Overall brucellosis prevalence was 17.92% (174/971, 95% CI: 15.64-20.46%) based on the diagnostic cut off point for the agglutination test (1:20). The estimated seroprevalence was adjusted to 11.64% (n = 113) based on the recommended diagnostic cut off point of ≥ 1:80 for rapid agglutination tests. Prevalence of active infections among the tested individuals was 6.90% (67/971), based on the widely recommended diagnostic titre of ≥ 1:160 for brucellosis. Most positive cases (56.32%, n = 98) detected in this study were examined by physicians at private medical facilities, while the remainder were from public hospitals and clinics.

4.4.3. Prevalence of positive cases by year

Annual prevalence of seropositive patients (Figure 4.1) differed between 2012 and 2013 ($p = 0.04$), as well as 2016 and 2017 ($p = 0.015$), but not between 2013 and 2014 ($p = 0.11$), 2014 and 2015 ($p = 0.98$), or 2015 and 2016 ($p = 0.84$).

4.4.4. Positive reactors by age and gender

For each age category (Figure 4.2), there were proportionally more female seropositive cases (64.37%, 112/174) than male (35.63%, 62/174), except in the age categories 6-12, 51-60 and 61-84 years. Of the 113 patients confirmed as positive based on the recommended cut off titre of ≥ 1:80, 68.14% (77/113) were female, while 31.86% (36/113) were male. Overall, positive cases were clustered in the 20-

50 year age range, with the highest proportion of positive cases in the 30-40 year age group (32.33%, 58/174) and the lowest in the 0-5 year age group (0.57%, 1/174).

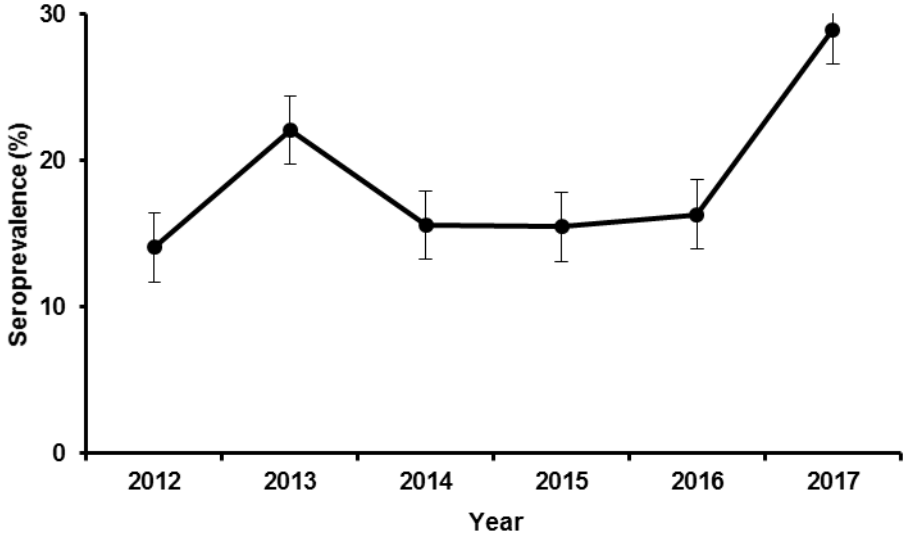


Figure 4.1: Seroprevalence of human brucellosis among the tested patients by year.

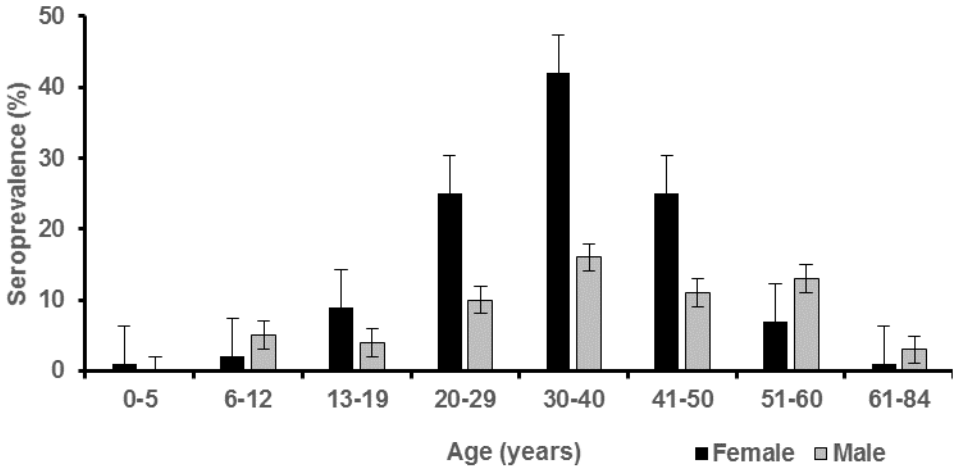


Figure 4.2: Seroprevalence of human brucellosis among suspected cases in Namibia by age and gender, 2012 - 2017.

The prevalence of seropositive patients in both males and females increased with age from 0-5 years of age to reach peak prevalence at 30-40 years and declined thereafter (Figure 4.2). Annual and overall prevalence of anti-*Brucella* antibodies was

higher in females than in males (Figure 4.3), but the differences were not significant ($\chi^2 = 2.24$, $p = 0.14$).

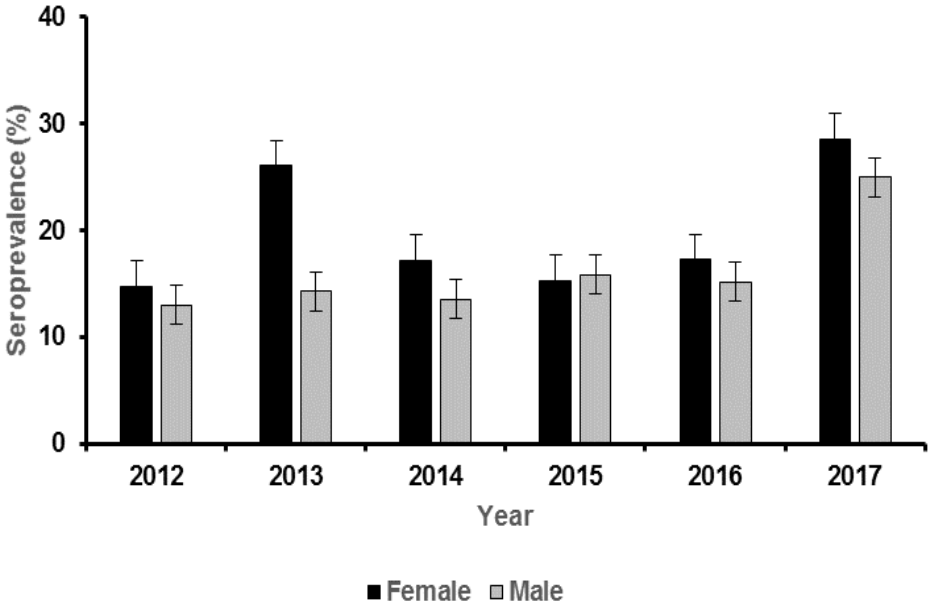


Figure 4.3: Seroprevalence of human brucellosis among suspected cases in Namibia by gender, 2012 – 2017.

4.4.5. Distribution of positive cases

A higher proportion of positive cases (55.17%, 96/174) were confirmed during the rainy season (October-March) than the dry season (April-Sept) (44.83%, 78/174), with November (n = 27) recording the highest number of positive cases. However, the seasonal differences in prevalence were not statistically significant ($z = 1.9$, $p = 0.05$).

All 14 regions of the country recorded at least one incident case of brucellosis (Figure 4.4) over the study period. The highest number of incident cases were in Khomas (n = 66), followed by Hardap (n = 41), Kunene (n = 17), Karas (n = 15), Oshana (n =11) and the rest of the regions (1 - 10 cases). Most regions where the human population lives on communal areas such as Kavango, Zambezi, Omusati, Ohangwena and Oshikoto, had lower incidence of the disease (1-4 cases) than regions with urban areas.

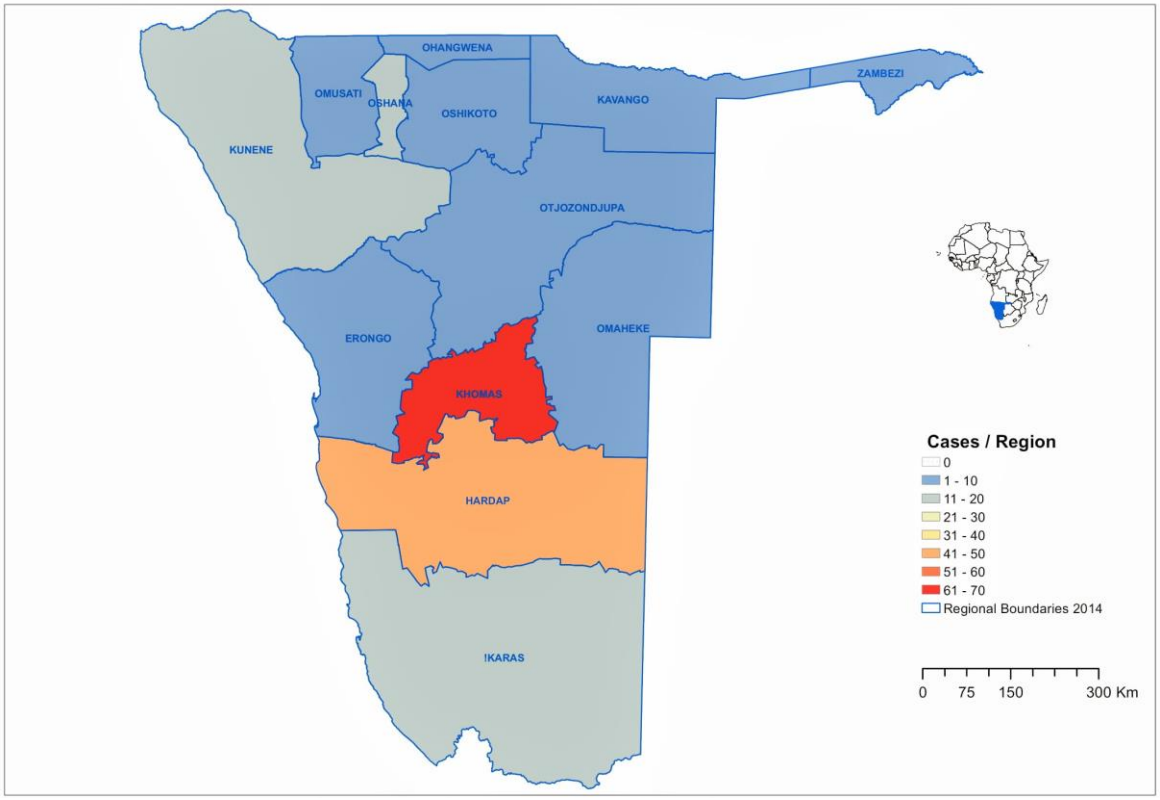


Figure 4.4: Number of incident cases reported per region from 2012-2017. The highest number of cases were recorded in the Khomas region followed by the Hardap region, with lower cases in the rest of the regions.

A log binomial regression model was used to determine the relative risk (RR) of infection for age and gender, using Oshikoto as the reference region. The risk of infection was significant in the Erongo region (RR: 0.398, 95% CI: 0.164 - 0.962) compared to Oshikoto and other regions. Neither age category (RR: 0.980 95% CI: 0.907 - 1.05) nor gender (RR: 0.800, 95% CI: 0.598 - 1.069) were significant predictors of risk of *Brucella* infection.

4.4.6. Incidence of brucellosis

Overall, the incidence of brucellosis over the six-year study period was 6.96 per 100 000 persons. This was calculated based on an average population of 2 500 000 people. Among administrative regions, relatively high incidence was recorded for the Hardap (44.27), followed by Karas (17.35), Kunene (15.49), and Khomas (14.11), while the other regions had incidence of 0.49-4.58 per 100 000 persons (Figure 4.5).

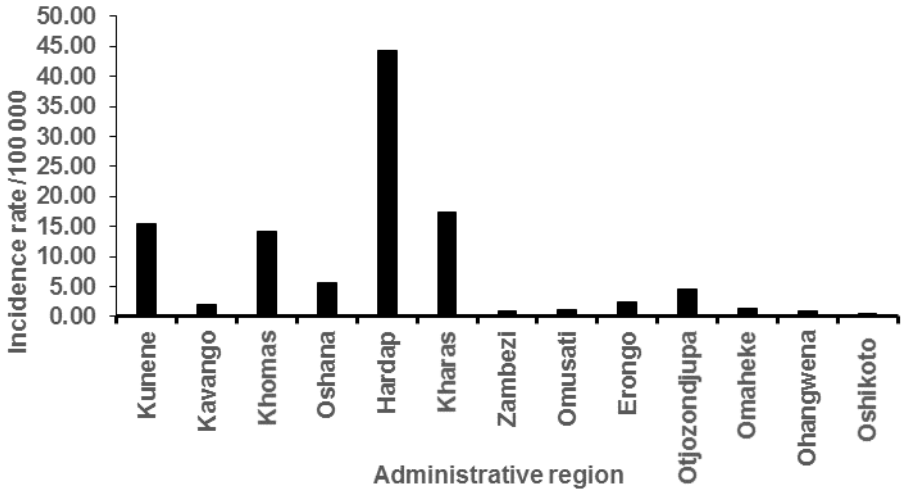


Figure 4.5: A comparison of incidence of brucellosis per 100 000 persons among the administrative regions of Namibia. Hardap recorded the highest incidence.

4.5. Discussion

Brucellosis is a zoonotic disease with the potential to cause serious impacts on the health of a population. The prevalence of the disease in the animal population typically corresponds with the prevalence in the human population (Refai, 2002; WHO, 2006; Makita et al., 2010; Osoro et al., 2015). Human brucellosis cases are a good indicator of the presence of infection in animal populations (WHO, 2006). Control of the disease in animals often results in reduced incidence in humans. In Namibia, brucellosis is a notifiable and endemic disease that has been reported in cattle, sheep, and goat populations over the years (Magwedere et al., 2011; Madzingira and McCrindle, 2014; 2015; 2016; Madzingira and Sezuni, 2017).

In this study, we document an apparent serological prevalence (symptoms and serological diagnosis) of 11.64% for brucellosis among patients of varied ages that presented with symptoms suggestive of human brucellosis in Namibia from 2012 to 2017. Since seropositive patients were 5 years and older, congenital infection is unlikely to have played a role. In contrast to the high seroprevalence (11.64%) determined in humans in this study, studies in cattle and sheep (the most likely sources for human infection), have consistently yielded prevalence of less than 2% over the years (Magwedere et al. 2011; Madzingira and McCrindle, 2014; Madzingira

and Sezuni, 2017). Therefore, the possibility exists that the testing of persons with brucellosis-like symptoms could have exaggerated the seroprevalence in this study. As expected, the prevalence determined in this study was higher than the 2.19% reported in a sample of apparently healthy abattoir workers in Namibia (Magwedere et al., 2011). It was comparable to a prevalence of 17% reported in Uganda (Tumwine et al., 2015), but lower than the 23.3% reported in South Sudan (Madut et al., 2018). Previous studies that were based on serological testing of presumptive clinical cases and made use of a similar commercial rapid slide agglutination test as in this study, reported prevalence of 12.8% (Alkahtani et al., 2020) and 19.6% (de Glanville et al., 2017). Understandably, studies involving patients with brucellosis-like symptoms, but using serological tests of a higher sensitivity and specificity, have reported lower prevalence of 4.7% (Mutanda, 1998), 2.6% (Animut et al., 2009) and 6% (Garifita et al., 2017). As per the case definition, presumptive cases with a positive serological test were considered as confirmed or positive brucellosis cases and placed on a standard six-week treatment course of a combination of doxycycline and rifampicin as per the WHO recommended treatment protocol (WHO, 2006). Presumptive patients with a seronegative test probably had other infections that are prevalent in the country such as malaria and typhoid that manifest clinical symptoms similar to brucellosis (Maichomo et al., 1998; 2000).

Some authors recommend a diagnostic cut-off of 1:80 for rapid agglutination tests (Sathyanarayan et al., 2011), while others consider a titre of ≥1:160 as diagnostic of active brucellosis (Rodríguez et al., 1987**;** Young, 1991) especially in patients presenting with symptoms suggestive of brucellosis (WHO, 2006). Taking the two cut-off points into consideration, the estimate of seroprevalence in this study was 11.64% (≥1:80) and the prevalence of active infections was 6.8% (≥1:160), which are comparable to results of studies from other African countries (Mutanda, 1998; Gafirita et al., 2017). However, since patients tested in this study had brucellosis–like symptoms, positive titres can be considered with confidence as confirming disease in the tested individuals.

Although brucellosis cases were identified in all age groups, positive cases were clustered in the 20-50 year age group, with the highest infection rate in the 30-40

year age group, confirming findings by previous studies (Al-Tawfiq and AbuKhamsin, 2009; Al-mashhadany, 2018; Lakew et al., 2019). The 20-50 year age group comprises the most active and working part of the any country's population, which is likely to be exposed to infection through activities such as assisting with delivery, milking, and slaughtering. This study also confirms previous reports by Lulu et al. (1988) that brucellosis incidence is low in the young, perhaps due to less contact with animals compared to adults (Alkahtani et al., 2020).

The majority of confirmed brucellosis cases were females as has also been reported by de Glanville et al. (2017). The predominance of female cases may reflect the roles played by females, which increase exposure to *Brucella* infection such as milk processing, home cheese preparation and working in the meat industry. However, this could also reflect the structure of the study sample and the Namibia population, where females were in the majority (51.60%) (World Bank, 2019). Reports from around the world indicate that the distribution of brucellosis cases is not gender-specific (Al Dahouk et al., 2007; Lakew et al., 2019), but that behavioral practices are the major determinant of exposure and disease burden (Bikas et al., 2003).

Brucellosis cases were recorded in all 14 administrative regions of the country. Most positive cases (79.89%) were in the major sheep farming regions (Khomas, Hardap, Karas) and a region (Kunene) where pastoralism is practised. Therefore, brucellosis strategies should be focused on these regions to decrease national disease incidence as sheep and goats are major sources of *Brucella* infection for humans worldwide (Feng, 1992, Memish, 2001, Dogany and Aygen, 2003). Surprisingly, fewer cases of the disease were reported in most regions where livestock rearing is communal. Whether this observation is due to under-reporting because of limited access to medical facilities (Kunda et al, 2007) or confusion with endemic diseases, such as malaria (Animut et al., 2009), that are prevalent in these regions, is not immediately clear and is a subject for future studies. However, reports from other countries point to a high incidence of *Brucella* infection in communal pastoral systems than other systems (Hamdy and Amin, 2002).

It was encouraging that suspected cases of brucellosis decreased from 2012 to 2017, concurrent with a decrease in brucellosis cases in cattle and sheep in Namibia (Madzingira and McCrindle, 2014; 2015; 2017) due to the enforcement of the requirement for compulsory vaccination of heifers at 3-8 months of age with *B. abortus* S19 vaccine in the country.

Based on a relatively high prevalence of human brucellosis recorded in this study, a strategy with measures to prevent and control the disease in both humans and animals is recommended. The measures, preferably involving a one health approach, should include education and awareness campaigns to disseminate knowledge on risk factors for the disease to the public, medical and occupationally exposed professions such as butchers and farmers. Occupationally exposed professionals should consider minimizing direct contact with animals or their fluids by using effective protective gear such as gloves. Pasteurization or boiling of milk is a well-documented measure for reducing the incidence of human brucellosis (Kiambi et al., 2020). Control of animal brucellosis based on vaccination, movement controls, testing and culling as stipulated in the Animal Health regulations (2018) is an effective approach for controlling the disease in Namibia (Madzingira et al., 2020).

The study had its limitations. The rapid agglutination assay used in this study has lower sensitivity (36.6%) and specificity (69.3%) than other tests (RBT, Coombs and the Lateral Flow Assays) (Kiambi et al., 2020), which can lead to overestimation of brucellosis prevalence, especially in endemic areas and in regions with low prevalence (de Glanville et al. 2017; Kiambi et al., 2020). It is recommended that the test be used in combination with another test of higher specificity such as the RBT, SAT or Coombs test. The absence of a full clinical history for each patient precluded the complete assessment of clinical symptoms, exposure, and risk factors. Furthermore, the infecting *Brucella* species could not be identified using serological tests (Ducrotoy et al., 2016). Therefore, the isolation of the infecting *Brucella* species is recommended in future studies.

4.6. Conclusions

The study determined a relatively high prevalence of anti-*Brucella* antibodies in the tested patients, with cases reported in all regions of the country. Although age, gender and geographical location were associated with disease prevalence, neither was significant risk factors for *Brucella* seropositivity.

4.7. References

AL DAHOUK, S., NEUBAUER, H., HENSEL, A., SCHONBERG, I., NOCKLER, K., ALPERS, K., MERZENICH, H., STARK, K. & JANSEN, A. 2007. Changing epidemiology of human brucellosis, Germany, 1962-2005. *Emerging Infectious Disease*, 13, 1895-1900. DOI: 10.3201/eid1312.070527.

AL-MASHHADANY, D.A. 2018. Application of Rose Bengal test for surveillance of human brucellosis in Erbil Governorate Kurdistan Region Iraq. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 3, 162-175. DOI: 10.26479/2018.0401.14.

AL-TAWFIQ, J.A. & ABUKHAMSIN, A. 2009. A 24-year study of the epidemiology of human brucellosis in a health-care system in Eastern Saudi Arabia. *Journal of Infection and Public Health*, 2, 81-85. DOI: 10.1016/j.jiph.2009.03.003.

ANIMUT, A., MEKONNEN, Y., SHIMELIS, D, & EPHRAIM, E. 2009. Febrile illnesses of different etiology among outpatients in four health centres in northwestern Ethiopia. *Japanese Journal of Infectious Diseases*, 62, 107-110.

BERGER, S. 2016. Brucellosis: global status. Los Angeles: GIDEON Informatics Inc.

BIKAS, C., JELASTOPULU, E., LEOTSINIDIS, M. & KONDAKIS, X.L. 2003. Epidemiology of human brucellosis in a rural area of North-Western Peloponnese in Greece. *European Journal of Epidemiology,* 18, 267-274. DOI: 10.1023/A:1023368420840.

BOSCHIROLI, M.L., FOULONGNE, V. & O'CALAGHAN, D. 2001. Brucellosis: a worldwide zoonosis. *Current Opinion in Microbiology*, 4, 58–64. DOI: 10.1016/s1369-5274(00)00165-x.

CHUKWU, C.C. 1987. Brucellosis in Africa, Part II: The importance. *Bulletin of Animal Health and Production in Africa,* 35, 92-98.

DE GLANVILLE, W.A., CONDE-ÁLVAREZ, R., MORIYÓN, I., NJERU, J., DIÁZ, R., COOK, E.A.J., MORIN, M., BRONSVOORT, B.M.C., THOMAS, L.F., KARIUKI, S. & FÈVRE, E.M. 2017. Poor performance of the rapid test for human brucellosis in health facilities in Kenya. *PLoS Neglected Tropical Diseases*, 11, e0005508. DOI: 10.1371/ journal.pntd.0005508.

DOGANY, M. & AYGEN, B. 2003. Human brucellosis: an overview. *International Journal of Infectious Diseases,* 7, 173-182. DOI: 10.1016/S1201-9712(03)90049-X.

DUCROTOY, M.J., CONDE-AÍVAREZ, R., BLASCO, J.M. & MORIYÓN, I. 2016. A review of the basis of the immunological diagnosis of ruminant brucellosis. *Veterinary Immunology and Immunopathology*, 171, 81–102. DOI: 10.1016/j.vetimm.2016.02.002.

EC (European Commission). 2001. Brucellosis in sheep and goats (*Brucella melitensis*). Brussels: Health and Consumer Protection Directorate General, European Union. https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out59_en.pdf. Accessed June 18, 2018.

FALLATAH, S.M., ODULOJU, A.J., AL-DUSARI, S.N. & FAKUNLE, Y.M. 2005. Human brucellosis in Northern Saudi Arabia. *Saudi Medical Journal,* 26, 1562-1566.

FAO/WHO. 1986. Joint FAO/WHO expert committee on brucellosis. World Health Organization Technical Report Series 740. Geneva: WHO. p 1-132. https://apps.who.int/iris/handle/10665/40202. Accessed June 25, 2019.

FENG, J.L. 1992. Control of animal brucellosis in China. In: Strategies in diagnosis and control of brucellosis in Asia. Beijing.

FENSTERBANK, R. 1986. Brucellosis in cattle, sheep and goats: diagnosis, control and vaccination. *Revue scientifique et technique*, 5, 605-618. DOI: 10.20506/rst.5.3.269.

FOSTER, J.T., WALKER, F.M., RANNALS, B.D., HUSSAIN, M.H., DREES, K.P., TILLER, R.V., HOFFMASTER, A.R., AL-RAWAHI, A., KAIM, P. & SAQIB, M. 2018. African lineage *Brucella melitensis* isolates from Omani livestock. *Frontiers in Microbiology*, 8, 2702. DOI: 0.3389/fmicb.2017.02702.

FOURNIER, A., YOUNG, I., RAJIC, A., GREIG, J. & LEJEUNE, J. 2015. Social and economic aspects of the transmission of pathogenic bacteria between wildlife and food animals: a thematic analysis of published research knowledge. *Zoonoses and Public Health,* 62, 417–428. DOI: 10.1111/zph.12179.

FSAI (Food Safety Authority of Ireland). 2009. Health risks from unpasteurized milk. Dublin; 2009. http://www.fsai.ie. Accessed June 25, 2018.

GAFIRITA, J., KIIZA, G., MUREKATETE, A., NDAHAYO, L.L., TUYISENGE, J., MASHENGESHO, V., RUHIRWA, R., NYANDWI, T., ASIIMWE-KATEERA, B., NDAHINDWA, V. & NJUNWA, K.J. 2017. Seroprevalence of brucellosis

among patients attending a district hospital in Rwanda. *American Journal of Tropical Medicine and Hygiene,* 97, 831-835. DOI: 10.4269/ajtmh.16-0632.

GODFROID, J. 2002. Brucellosis in wildlife. *Revue Scientifique et Technique*, 21, 277-286. DOI: 10.20506/rst.21.2.1333.

GODFROID, J., CLOECKAERT, A., LIAUTARD, J.P., KOHLER, S., FRETIN, D., WALRAVENS, K., GARIN-BASTUJI, B. & LETESSON, J.J. 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Veterinary Research,* 36, 313-26. DOI: 10.1051/vetres:2005003.

GODFROID, J., SCHOLZ, H.C., BARBIER, T., NICOLAS, C., WATTIAU, P., FRETIN, D., WHATMORE, A.M., CLOECKAERT, A., BLASCO, J.M., MORIYON, I., SAEGERMAN, C., MUMA, J.B., AL DAHOUK, S., NEUBAUER, H. & LETESSON, J.J. 2011. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive Veterinary Medicine*, 102, 118-131. DOI: 10.1016/j. pretvetmed.2011.04.007.

GODFROID, J., AL DAHOUK, S., PAPPAS, G., ROTH, F., MATOPE, G., MUMA, J., MARCOTTY, T., PFEIFFER, D. & SKJERVE, E.A. 2013. A “One Health” surveillance and control of brucellosis in developing countries: moving away from improvisation. *Comparative Immunology, Microbiology and Infectious Diseases*, 36, 241–248. DOI: 10.1016/j.cimid.2012.09.001.

HAMDY, E.M.R. & AMIN, A.S. 2002. Detection of *Brucella* species in the milk of infected cattle, sheep, goats, and camels by PCR. *The Veterinary Journal*, 163, 299-305. DOI: 10.1053/tvjl.2001.0681.

HOTEZ, P.J. & GURWITH, M. 2011. Europe's neglected infections of poverty. *International Journal of Infectious Diseases*, 15, e611-619. DOI: 10.1016/j.ijid.2011.05.006.

INFO-NAMIBIA. 2019. Namibia-General information. https://www.info-namibia.com/info/general-information. Accessed January 10, 2019.

KIAMBI, S.G., FÈVRE, E.M., OMOLO, J., OUNDO, J. & DE GLANVILLE, W.A. 2020. Risk factors for acute human brucellosis in Ijara, north-eastern Kenya. *PLoS Neglected Tropical Diseases*, 14(4), e0008108. DOI: 10.1371/journal.pntd.0008108.

KUNDA, J., FITZPATRICK, J., KAZWALA, R., FRENCH, N.P., SHIRIMA, G.,

MACMILLAN, A., KAMBARAGE, D., BRONSVOORT, M. & CLEAVELAND, S. 2007. Health-seeking behaviour of human brucellosis cases in rural Tanzania. *BMC Public Health*, 7, 315. DOI: 10.1186/1471-2458-7-315.

KUPLULU, O. & SARIMEHMETOGLU, B. Isolation and identification of *Brucella* spp. in ice cream. *Food Control*, 15, 511-514. DOI: 10.1016/j.foodcont.2003.08.002.

KÜSTNER, H.G.V. 1985. Brucellosis in eastern Orange Free State. *Epidemiology Comments*, 12, 2-23.

LAKEW, A., HIKO, A., ABRAHA, A. & HAILU, S.M. 2019. Sero-prevalence and community awareness on the risks associated with livestock and human brucellosis in selected districts of Fafan Zone of Ethiopian-Somali National Regional State. *Veterinary and Animal Science*, 7, 100047. DOI: 10.1016/j.vas.2019.100047.

LECLERC, V., DUFOUR, B., LOMBARD, B., GAUCHARD, F., GARIN-BASTUJI, B., SALVAT, G., BRISABOIS, A., POUMEYROL, M., DE BUYSER, M.L., GNANOU-BESSE, N. & LAHELLEC, C. 2002. Pathogens in meat and milk products: surveillance and impact on human health in France. *Livestock Production Science*, 76, 195–202. DOI: 10.1016/S0301-6226(02)00126-4.

LUCERO, N.E., AYALA, S.M., ESCOBAR, G.I &, JACOB, N.R. 2007. The value of serologic tests for diagnosis and follow up of patients having brucellosis. *American Journal of Infectious Disease*, 3, 27–35. DOI: 10.3844/ajidsp.2007.27.35.

LULU, A.R., ARAJ, G.F., KHATEEB, M.I., MUSTAFA, M.Y., YUSUF, A.R. & FENECH, F.F. 1988. Human brucellosis in Kuwait: a prospective study of 400 cases. *Quarterly Journal of Medicine*, 66(249), 39–54.

MADUT, N.A., NASINYAMA, G.W., MUMA, J.B., SUBE, K.L.L., OCAN, M., MUWONGE, A., GODFROID, J., JUBARA, A.S., KANKYA, C. 2018. Prevalence of brucellosis among patients attending Wau Hospital, South Sudan. *PLoS One*, 13, e0199315. DOI: 10.1371/journal.pone.0199315.

MADZINGIRA, & MCCRINDLE, C. 2014. Prevalence of *Brucella* antibodies in sheep and springbok (*Antidorcas marsupialis*) reared together in the Karas region, Namibia. *Bulletin of Animal Health and Production in Africa*, 62, 299-306.

MADZINGIRA, & MCCRINDLE, C. 2015. Retrospective analysis of the prevalence of

Brucella antibodies in sheep in the Karas Region of Namibia. *Tropical Animal Health and Production*, 47, 1117. DOI: 10.1007/s11250-015-0838-z. 2015.

MADZINGIRA, & MCCRINDLE, C. 2016. A questionnaire survey of risk factors of brucellosis on mixed sheep and springbok (*Antidorcas marsupialis*) farms. *International Science and Technical Journal of Namibia*, 8, 43-49.

MADZINGIRA, & SEZUNI M.P. 2017. Serological prevalence and public health significance of brucellosis on a dairy farm in Namibia from 2011-2014. *BMC Research Notes*, 10, 620. DOI: 10.1186/s13104-017-2933-x.

MAGWEDERE, K., BISHI, A., TJIPURA-ZAIRE, G., EBERLE, G., HEMBERGER, Y., HOFFMAN, L.C. & DZIVA, F. 2011. Brucellae through the food chain: the role of sheep, goats and springbok (*Antidorcus marsupialis*) as sources of human infections in Namibia. *Journal of the South African Veterinary Association*, 82, 205-212. DOI: 10.4102/jsava.v82i4.75.

MAICHOMO, M.V.V., MCDERMOTT, J.J., ARIMI, S.M., GATHURA, P.B., MUGAMBI T, & MURIUKI, S.M. 2000. Study of brucellosis in a pastoral community and evaluation of the usefulness of clinical signs and symptoms in differentiating it from other flu-like diseases. *African Journal of Health Sciences*, 7, 114-119.

MAICHOMO, M.W., MCDERMOTT, J.J., ARIMI, S.M. & GATHURA, P.B. 1988. Assessment of the Rose Bengal Plate test for the diagnosis of human brucellosis in health facilities in Narok District, Kenya. *East African Medical Journal*, 4, 75.

MAKITA, K., FÈVRE, E.M., WAISWA, C., KABOYO, W., DE CLARE BRONSVOORT, B.M., EISLER, M.C. & WELBURN, S.C. 2008. Human brucellosis in urban and peri-urban areas of Kampala, Uganda. *Annals of the New York Academy of Sciences*, 1149, 309-311. DOI: 10.1196/annals.1428.015.

MAKITA, K., FEVRE, E.M., WAISWA, C., EISLER, M.C. & WELBURN, S.C. 2010. How human brucellosis incidence in urban Kampala can be reduced most efficiently? A stochastic risk assessment of informally-marketed milk. *PLoS One*, 5, 1–10. DOI: 10.1371/journal.pone.0014188.

MEMISH Z. 2001. Brucellosis control in Saudi Arabia: prospects and challenges. *Journal of Chemotherapy*, 12, 11-17. DOI: 10.1080/1120009x.2001.11782322

MINAS, M., MINAS, A., GOURGULIANIS, K. & STOUMARA, A. 2007.

Epidemiological and clinical aspects of human brucellosis in Central Greece. *Japanese Journal of Infectious Diseases*, 60, 362-366.

MORENO, S., ARIZA, J., ESPINOSA, F.J., PODZAMCZER, D., MIRÓ, J.M., RIVERO, A., RODRÍGUEZ-ZAPATA, M., ARRIZABALAGA, J., MATEOS, R. & HERRERO, F. 1988. Brucellosis in patients infected with the human immunodeficiency virus. *European Journal of Clinical Microbiology and Infectious Disease*, 17, 319-26. DOI: 10.1007/BF01709454.

MOYER, N.P. & HOLOCOMB, L.A. 2005. Brucella. In: MURRAY, P.R., JO BARRON, E., PFALLER, M.A., TENOVER, F.C., YOLKEN, R.H., editors. Manual of Clinical Microbiology, 6th edition. Washington DC: ASM Press; p. 549-555.

MUSALLAM, I.I., ABO-SHEHADA, M.N., HEGAZY, Y.M., HOLT, H.R. & GUITIAN, F.J. 2016. Systematic review of brucellosis in the Middle East: disease frequency in ruminants and humans and risk factors for human infection. *Epidemiology and Infection*, 144, 671–685. DOI: 10.1017/S0950268815002575.

MUTANDA, L. 1988. Selected laboratory tests in febrile patients in Kampala, Uganda. *East African Medical Journal*, 75, 68–72.

OSORO, E.M., MUNYUA, P., OMULO, S., OGOLA, E., ADE, F., MBATHA, P., MBABU, M., NG'ANG'A, Z., KAIRU, S., MARITIM, M., THUMBI, S.M., BITEK, A., GAICHUGI, S., RUBIN, C., NJENGA, K. & GUERRA, M. 2015. Strong association between human and animal *Brucella* seropositivity in a linked study in Kenya, 2012–2013. *American Journal of Tropical Medical and Hygiene*, 93, 224–231. DOI: 10.4269/ajtmh.15-0113.

PAPPAS, G., AKRITIDIS, N., BOSILKOVSKI, M. & TSIANOS, E. 2005. Brucellosis. *New England Journal of Medicine*, 352, 2325-2336. DOI: 10.1056/NEJMra050570.

PAPPAS, G.S., PAPADIMITRIOU, P., AKRITIDIS, N., CHRISTOU, I. & TSIANOS, E.V. 2006. The new global map of human brucellosis. *Lancet Infectious Disease*, 6, 91-99. DOI: 10.1016/S1473-3099(06)70382-6.

PAPPAS, G. 2010. The changing *Brucella* ecology: novel reservoirs, new threats. *International Journal of Antimicrobial Agents,* 36(Suppl 1), S8-11. DOI: 10.1016/j.ijantimicag.2010.06.013.

REFAI, M. 2002. Incidence and control of brucellosis in the Near East region. *Veterinary Microbiology*, 90, 81-110. DOI: 10.1016/S0378-1135(02)00248-1.

RENUKARADHYA, G.J., ISLOOR, S. & RAJASEKHAR, M. 2002. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. *Veterinary Microbiology*, 90, 183-195. DOI: 10.1016/s0378-1135(02)00253-5.

RODRÍGUEZ, T.A. & FERMOSO, J. 1987. Brucellosis. *Medicine*, 76, 3165-3177.

SATHYANARAYAN, M.S., SURESH, D.R., SONTH, S.B., KRISHNA, S., MARIRAJ, J., SUREKHA, Y.A., PRAKASH, R. & RAVIKUMAR, R.A. 2011. A comparative study of agglutination tests, blood culture and ELISA in the laboratory diagnosis of human brucellosis. *International Journal of Biology and Medical Research*, 2, 569–572.

SELEEM, M.N., BOYLE, S.M. & SRIRANGANATHAN, N. 2010. Brucellosis: a re-emerging zoonosis. *Veterinary Microbiology*, 140, 392-398. DOI: 10.1016/j.vetmic.2009.06.021.

STEHN, H. 2008. Large stock management. 2008. http://www.agrinamibia.com.na/wp-content/uploads/2018/02/3-Large-Stock-Manual.pdf. Accessed December 12, 2018.

TALESKI, V., ZERVA, L., KANTARDJIEV, T., CVETNIC, Z., ERSKI-BILJIC, M., NIKOLOVSKI, B., BOSNJAKOVSKI, J., KATALINIC-JANKOVIC, V., PANTELIADOU, A., STOJKOSKI, S. & KIRANDZISKI, T. 2002. An overview of the epidemiology and epizootiology of brucellosis in selected countries of Central and Southeast Europe. *Veterinary Microbiology*, 90, 147-155. DOI: 10.1016/s0378-1135(02)00250-x.

TUMWINE, G., MATOVU, E., KABASA, J.D., OWINY, D.O. & MAJALIJA, S. 2015. Human brucellosis: sero-prevalence and associated risk factors in agro-pastoral communities of Kiboga District, Central Uganda. *BMC Public Health*, 15, 900. DOI: 10.1186/s12889-015-2242-z.

WHO. 2006. Brucellosis in humans and animals. Geneva: WHO, FAO and OIE. 2006. https://www.who.int/csr/resources/publications/Brucellosis.pdf. Accessed August 16, 2019.

WORLD BANK. 2019. Population, female (% of total population). https://data.worldbank.org/indicator/SP.POP.TOTL.FE.ZS?view=chart. Accessed July 24, 2019.

YOUNG, E.J. 1991. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. *Review of Infectious Disease*, 13, 359-372. DOI: 10.1093/clinids/13.3.359.

ZHOU, L., FAN, M., HOU, Q., JIN, Z. & SUN, X. 2018. Transmission dynamics and optimal control of brucellosis in Inner Mongolia of China. *Mathematics, Bioscience and Engineering*, 15(2), 543–567. DOI: 10.3934/mbe.2018025.

ZINSSTAG, J., SCHELLING, E., ROTH, F., BONFOH, B., DE SAVIGNY, D. & TANNER, M. 2007. Human benefits of animal interventions for zoonosis control. *Emerging Infectious Disease*, 13, 527–531. DOI: 10.3201/eid1304.060381.

Chapter 5: A retrospective sero-epidemiological survey of bovine brucellosis on commercial and communal farming systems in Namibia from 2004-2018

Oscar Madzingira[1*], Folorunso Oludayo Fasina[2], Erick Kandiwa[1], Albertina Musilika-Shilongo[3], Frank Chitate[1,] Henriette van Heerden[4]

[1]School of Veterinary Medicine, Faculty of Agriculture and Natural Resources, University of Namibia, P. Bag 13301, Windhoek, Namibia
[2]Emergency Centre for Transboundary Animal Diseases-Food and Agriculture Organisation of the United Nations (ECTAD-FAO), Dar es Salaam
[3]Directorate of Veterinary Services, Ministry of Agriculture, Water and Forestry, P. Bag 12022, Government Office Park, Windhoek.
[4]Department of Veterinary Tropical Diseases, Room 2-11, Paraclinical Building University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

5.1. Abstract

Cattle production is the major livestock production activity and the mainstay of Namibia's economy. Sustained beef exports are contingent on a sound sanitary environment where diseases such as brucellosis are under control. In this retrospective study, 49 718 bovine brucellosis testing results from 2004 to 2018 were analysed to determine the proportion of seropositive cattle and herds, and the spatial distribution of positive reactors from commercial and communal areas. In total, 244 positive reactors were identified based on the Rose Bengal Test (RBT) and the Complement Fixation Test (CFT) in series, giving an overall proportion of infected animals of 0.49% (244/49718, 95% CI: 0.43-0.56%) and an overall proportion of infected herds of 9.26% (78/842, 95% CI: 7.49-11.41%). There was a higher proportion of seropositive communal herds (33.09%) and cattle (10.27%) than commercial herds (4.67%) and cattle (0.24%) ($p < 0.05$). Annually, the proportion of positive reactors was 0-1.37% in the commercial area and 0-52.38% in the communal areas, with a clear decline in positive reactors in the communal areas. Within the commercial sector, the proportion of positive reactor dairy, beef and export cattle was 0.19% (51/27067, 95% CI: 0.14-0.25%), 0.30% (48/16098, 95% CI:

0.22-0.40%) and 0.33% (16/4811, 95% CI: 0.20-0.54%) respectively. Abortions were found to be the major reason for *Brucella* testing in the communal areas. About 12.65% (96/759) of abortion-linked sera tested positive, but none were positive in beef or dairy cattle. Widespread vaccination of cattle and robust planned surveillance is recommended to reduce the incidence of the disease and its associated production losses and public health risk.

5.2. Introduction

Livestock production is a mainstay of the Namibian economy. Beef exports contribute approximately N$2.2 billion (US$157 million) annually, representing around 7 percent of Namibia's total annual export earnings (Mushendami et al., 2008). For sustained beef exports to niche markets, maintenance of a sound sanitary environment in which the major infectious and zoonotic diseases including, but not limited to bovine brucellosis are controlled is a prerequisite.

Bovine brucellosis is a highly infectious zoonotic disease of major public health and economic importance that affects humans and domestic animals in parts of the world (Alton, 1991). It is commonly caused by *Brucella abortus* or infrequently by *Brucella melitensis* or *B. suis* and is a disease of major concern in Sub-Saharan Africa (Corbel, 1997; McDermott and Arimi, 2002; OIE, 2018). Cattle to cattle transmission may occur through contact with infected material such as aborted fetuses, fetal membranes and vaginal secretions. Humans are accidental hosts with infection commonly acquired through the consumption of unpasteurized milk and dairy products such as soft cheeses. Veterinarians, farmers, butchers and laboratory personnel are always at greater risk of the disease due to regular contact with animals, their products (EC, 2001; Godfroid, 2002; WHO, 2005) and secretions.

Brucellosis is often overlooked and therefore underreported by public health and veterinary authorities in developing countries. As a result, it is considered a neglected zoonotic disease of poverty (Young, 1995; Hotez and Gurwith, 2011), as well as a re-emerging disease (Godfroid, 2002; Pappas, 2010). It contributes significantly to disease burden in livestock rearing areas of Africa (Fèvre et al., 2017). Large and mobile cattle herds that frequently intermingle with different herds

especially in communal pastoral systems, are at greater risk of the brucellosis (EC, 2001; McDermott and Arimi 2002; WHO, 2006). Other factors such as the type of production system (Cadmus et al., 2008), calving times (WHO, 2006), herd size and breed (Muma et al., 2007; Ndazigaruye et al., 2018) have also been associated with increased sero-positivity in cattle.

Diagnosis of brucellosis is primarily based on serological tests as they are cheap and readily available. According to McDermott et al. (2013), serological prevalence of bovine brucellosis in Africa ranges from 0-68%. In Namibia, the first official report of brucellosis was in 1953 in Karakul sheep (Godfroid et al., 2019). Thereafter, brucellosis has been reported in cattle, sheep and goats based on the clinical picture and scattered serological surveys (Magwedere et al., 2011; Madzingira and McCrindle, 2014; 2015; 2016; Madzingira and Sezuni, 2017). However, most of these reports were focused only on the commercial production system. In this system, cattle are raised on fenced farms and measures for the prevention of brucellosis such as biosecurity and vaccination are implemented. In the communal farming system, available grazing land is shared by a number of different cattle herds in the area.

To the best of our knowledge, no planned efforts to determine and compare the proportion of infected animals in different farming systems have been carried out in the country. Therefore, the aim of this study was to collate 15-year bovine brucellosis testing data and analyze it to determine the proportions of seropositive cattle and cattle herds in the communal farming system, commercial dairy and beef cattle farming systems, as well as to find out the spatial distributions of positive reactors. This information is necessary for a better understanding of the epidemiology of the disease in Namibia and to provide a sound basis for implementing targeted and effective prevention and control measures.

5.3. Materials and Methods

5.3.1. Study area

Namibia is located at -22°58'1.42"S and 18°29'34.80"E in the South-Western part of Africa. It covers a total land area of 824 292 km² (Info-Namibia, 2019) and is divided into 14 administrative regions. About 2.7 million herd of cattle are reared in both commercial and communal areas of Namibia. Daytime temperatures vary from around 20˚C at the coast to 50˚C in the desert. Namibia has two main seasons, summer or the rainy season (October to March) and winter or dry season (April to September). The Zambezi region receives the highest annual rainfall of about 700 mm, while the lowest annual rainfall is recorded in desert areas in the western coastal areas and the southern regions.

5.3.2. Study design

Secondary data, comprising of bovine brucellosis testing results of cattle in Namibia (January 2004 to December 2018 inclusive) was used in the study. The data was from the mandatory annual testing of dairy cattle; the testing of beef cattle suspected of the disease or destined for sales (export or local); communal area cattle suspected to have the infection and imported animals. The data was stratified into commercial (beef, dairy, exports and imports) and communal areas for analysis. In all cases, requests for testing for bovine brucellosis were initiated by or sent under the supervision of state veterinarians. Serological testing was carried out at the state laboratory, the Central Veterinary Laboratory (Windhoek) and the data was kept at the Epidemiology and Surveillance Section (Directorate of Veterinary Services, Ministry of Agriculture, Water and Land Reform).

5.3.3. Data collection

With the permission of the Chief Veterinary Officer, *Brucella* serological testing results from January 2004 to December 2018 inclusive were retrieved. The data comprised of results of suspected clinical cases that were tested for confirmation and results of dairy, beef, exported and imported cattle that were tested as part of routine surveillance for brucellosis in the commercial and communal farming areas of the country. The data set included the date of sampling, farm name, farm number, farm coordinates, owner, magisterial district, species, reason for testing, number of cattle

tested and the number of positive reactors. Age and sex were inconsistently recorded and were thus not taken into consideration during data analyses. Proportions of positive reactor cattle and cattle herds were determined per farming sector and year. The spatial distribution of positive cases was determined.

5.3.4. Testing of sera

The data used in this study was from bovine sera that were tested for antibodies to smooth *Brucella* species (*B. abortus, B. melitensis* and *B. suis*) at the Central Veterinary Laboratory in Windhoek using the Rose Bengal Test (RBT) as a screening test and the Complement Fixation Test (CFT) as a confirmatory test. The RBT and CFT tests were performed following the procedures and guidelines described by the OIE (2004). Any visible agglutination or clumps were considered as an indicator of a positive RBT test (OIE, 2004). All sera positive on RBT were subjected to confirmation using the CFT. For the CFT, titres of 1:8 and above were considered as positive based on the presence or absence of haemolysis. The RBT has been reported to have a specificity of 71-80% and a sensitivity of 78-100% (Díaz-Aparicio et al., 1994; Bercovich, 1998), while a specificity and a sensitivity of 98% and 81% respectively has been ascribed to the CFT (Bercovich, 1998).

5.3.5. Data analyses

Brucellosis testing results were stored in Microsoft Excel® spreadsheet version 2007 (Microsoft Corporation, Redmond, WA). Descriptive statistics (proportions) were determined for positive reactors both in the commercial and communal areas. The 95% confidence intervals (CI) for proportions were calculated taking into account CFT test sensitivity and specificity of 81% and 98% respectively, according to the method described by Reiczigel et al. (2010). Further statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 25. Proportions of serological reactors were compared across farming sector, year, and administrative regions. The *z*-score test was used to test for the significance of differences between two proportions. In all cases, $p < 0.05$ was considered significant.

5.3.6. Ethical approval

The study was approved by the Chief Veterinary Officer, Ministry of Agriculture, Water and Land Reform (Namibia) (Appendix 11), and the Research Ethics Committee (REC056-20) (Appendix 6) of the University of Pretoria.

5.4. Results

From 2004 to 2018, a total of 49 718 cattle sera, from the commercial (beef, dairy, export and imported animals, n = 48462) and communal farming sectors (n = 1256) were submitted to the Central Veterinary Laboratory tested for *Brucella* serology from 842 cattle herds (706 commercial and 136 communal cattle herds) (Table 5.1). The overall proportion of positive cattle and cattle herds over the 15-year was estimated at 0.49% (244/49718) and 9.26% (78/842) respectively. The percentage of reactor cattle (12.68%, 146/1151) and cattle herds (27.54%, 46/167) was higher in the communal areas than in the commercial areas (0.19%, 69/36192 and 4.30%, 26/605) ($p < 0.05$). A comparison of the different animal production sectors (Table 5.1) revealed that a higher proportion of positive herds (33.09%) and seropositive cattle (10.27%) were in the communal areas than in the commercial cattle farming sectors ($p < 0.05$). No differences in the overall proportions of infected cattle in the dairy, beef and export sectors were observed ($p < 0.05$).

Table 5.1: Proportions of *Brucella* positive cattle and herds in the commercial (beef, dairy, export) and communal farming sectors of Namibia, 2004-2018.

Farming sector		Number of farms/ herds tested	Number of farms/ herds positive	% positive farm/ herds	Number of sera tested	Number of sera positive	% positive sera (95% CI)
Commercial							
area	Beef	514	11	2.14 (1.20-3.79)	16098	48	0.30(0.22-0.40)
	Dairy	133	17	12.78 (8.14-19.52)	27067	51	0.19 (0.14-0.25)
	Export	51	5	9.80 (4.26-20.98)	4811	16	0.33 (0.20-0.54)
	Imports	8	0	0 (0)	486	0	0 (0)
	Total	706	33	4.67 (3.35-6.49)	48462	115	0.24 (0.20-0.28)
Communal area		136	45	33.09 (25.74-41.37)	1256	129	10.27 (8.71- 2.07)
	Overall	**842**	**78**	**9.26 (7.49-11.41).**	**49718**	**244**	**0.49 (0.43-0.56)**

An average of 47.07 (23-80) and 9.07 (1-17) cattle herds were tested annually in the commercial and communal farming sectors respectively giving a proportion of positive cattle herds of 0-12.77% in the commercial areas and 0-52.38% in the communal areas. Approximately 1 in every 21 farms (33/706) tested in the commercial areas, and 1 in every 3 farms (45/136) tested in the communal areas was positive for brucellosis. The mean number of cattle sera tested per year in the commercial and communal areas was 3230.80 (1149-8962) and 83.73 (1-266) respectively (Table 5.2). The annual percentage of positive reactors showed a decreasing trend and varied from 0-1.37% and 0-52.38% in the commercial and communal areas respectively.

Table 5.2: Number of sera tested and proportions of sera positive per year of study.

Year	Sera tested: commercial areas	Number positive (% positive)	Sera tested: communal areas	Number positive (% positive)
2004	3456	19 (0.55)	56	17 (30.36)
2005	2745	2 (0.07)	42	0 (0)
2006	3374	13 (0.39)	21	11 (52.38)
2007	2128	0 (0)	4	0 (0)
2008	1865	7 (0.38)	60	8 (13.33)
2009	1384	19 (1.37)	266	3 (1.13)
2010	3213	0 (0)	79	7 (8.86)
2011	3055	0 (0)	85	2 (2.35)
2012	2520	16 (0.63)	106	18 (16.98)
2013	2847	2 (0.07)	204	40 (19.61)
2014	3123	1 ((0.03)	131	17 (12.98)
2015	1149	6 (0.52)	152	0 (0)
2016	8962	1 (0.01)	14	0 (0)
2017	4176	14 (0.34)	2	0 (0)
2018	4465	15 (0.34)	1	0 (0)

Overall, it was 10.08 (95% CI: 6.12 -16.62) times more likely for farms from the communal areas to test positive for brucellosis than those from the commercial areas (Table 5.3). The overall odds of an animal in the communal areas testing positive for

Brucella antibodies were 48.12 (95% CI: 37.17- 62.30) times compared to an animal in the commercial areas.

Table 5.3: Overall odds of brucellosis on farms during the study period.

Sector	Number of farms tested	Number of farms positive	Odds Ratio (Communal vs Commercial)	95% CI
Communal	136	45	10.08	6.12 - 16.62
Commercial	706	33		

The number and proportions of seropositive cases detected by the RBT and CFT per sector are depicted in Table 5.4. Overall, 62.89% (244/388) of the CFT results agreed with the RBT. Therefore, the RBT was associated with 37.11% (144/388) false positive results. The CFT titre for positive reactors ranged from 1:8 to 1:256.

Table 5.4: Positive reactors detected by the RBT and CFT per sector.

Sector	Number positive on RBT	Number positive on CFT (% of RBT)
Commercial area	232	115 (49.57)
Communal area	156	129 (82.69)
Total	388	244 (62.89)

Sampling and serological testing for *Brucella* antibodies in the communal areas was primarily initiated by reports of cow abortions. As indicated in Table 5.5, 77.20% (105/136) of the farms and 60.43% (759/1256) of the sera were tested for *Brucella* antibodies following reports of abortions. No reports of abortions were recorded in dairy cows over the study period, while in beef cattle, all sera (n = 252) that were associated with abortions were seronegative. Of all the positive cases identified in the communal areas (n = 129), 74.42% (n = 96) were linked to abortions. Cattle that were reported to have hygromas or orchitis tested negative for *Brucella* antibodies. In addition, brucellosis was also suspected and tested for based on the observation of retained placentas and neonatal mortality in the beef, dairy, and communal farming sectors.

Table 5.5: Number and proportion of abortion cases reported in the three farming sectors from 2004-2018. All abortion linked sera from beef cattle were negative for brucellosis, while 12.65% of abortion-linked sera from communal cattle tested positive for brucellosis.

Cattle farming sector	Number of farms tested due to abortions	Number of positive farms (%)	Number of sera tested due to abortions	Number of sera positive (%)
Beef cattle	42	0	252	0
Dairy cattle	0	0	0	0
Communal cattle	105	35 (33.33)	759	96 (12.65)
Total	**147**	**35 (23.80%)**	**1011**	**96 (9.50%)**

The spatial distribution of positive reactors per region is shown in Figure 5.1. *Brucella* positive reactors were clustered in the communal areas (Zambezi, Kavango, and the North Central Regions (NCR)), with focal areas of infection in commercial farming regions (Otjozondjupa, Khomas and Hardap regions). Positive dairy farms were confined to four out of the 14 administrative regions of the country, namely, Karas (n = 1), Hardap (n = 4), Khomas (n = 5) and Otjozondjupa (n = 3).

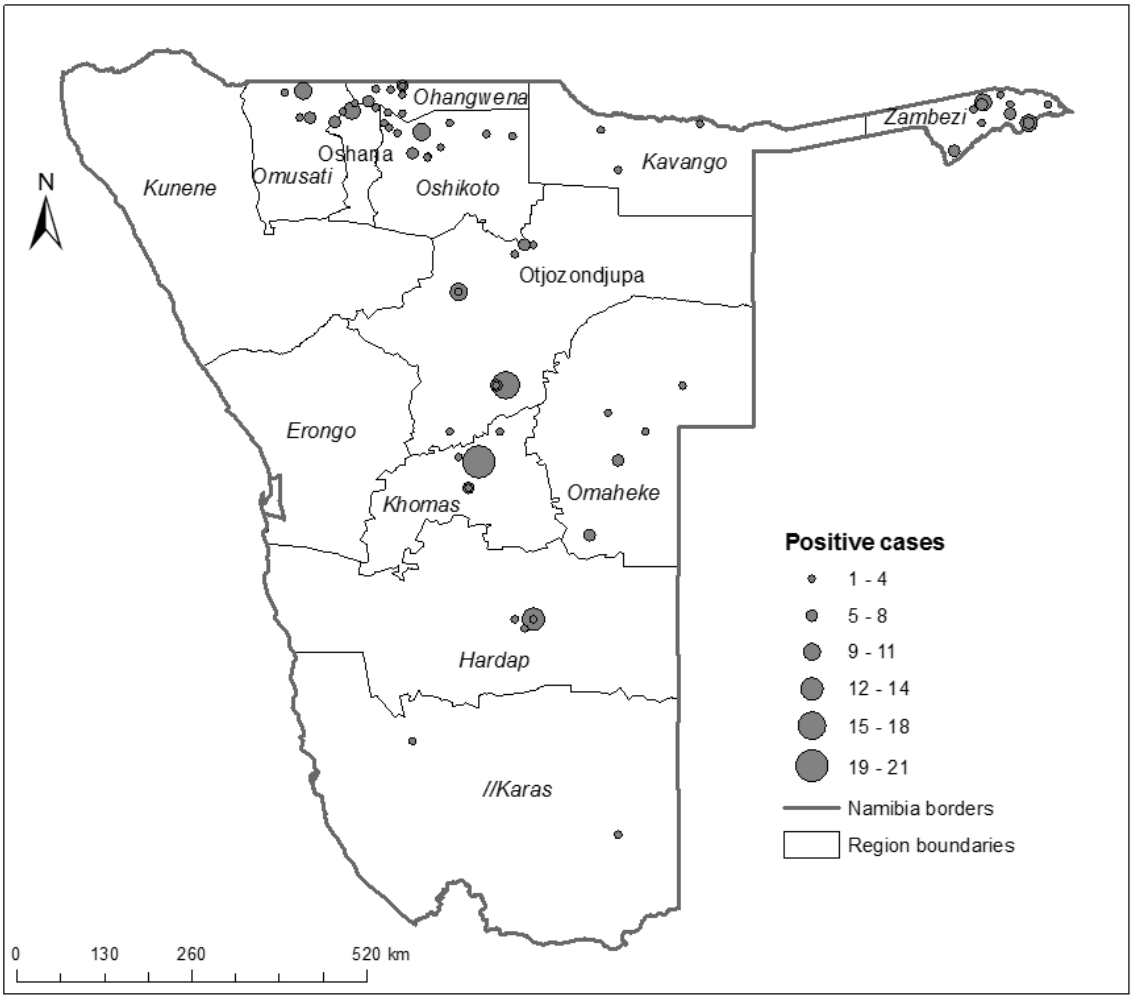


Figure 5.1: Map of Namibia showing the distribution of seropositive cattle. Cases were clustered in the northern (Omusati, Oshana, Ohangwena and Oshikoto) and north-eastern (Zambezi) regions of the country in which cattle production is under a communal system.

5.5. Discussion

Brucellosis is a notifiable disease in Namibia. Measures for the prevention and control of the disease are prescribed in the Animal Health Regulations (2018) to prevent negative impacts on animal and public health and international markets for beef. Continuous clinical or serological surveillance for the disease is one of the strategies utilized for early detection and control of the disease in the country.

In this 15-year retrospective study, the overall proportion of individual reactor cattle and cattle herds was 0.49% and 9.26% respectively. Previous studies have reported that individual animal and herd prevalence of less than 10% and 35% respectively,

as determined in our study, indicates a low prevalence of brucellosis in cattle (WHO, 1965). The proportion of positive reactor cattle (0.49%) determined by this study is like a prevalence of 0.5% (DVS, 1987) and within the range of 0-1.94% (Magwedere et al., 2011) reported by earlier studies in Namibia. Although over 97.47% of the cattle and 83.85% of the herds sampled in the study originated from the commercial areas, there was a higher proportion of reactor cattle and herds in the communal than commercial areas. The much higher proportion of *Brucella* positive cattle observed in the communal areas is a cause for concern and needs to be examined more closely. Reasons for the high proportion may be due to a surveillance system that specifically targeted suspected clinical cases and the absence of robust testing of herds; a cattle management system that favours the mingling of different herds and the transmission of brucellosis within and between herds; and a lack of awareness of the disease among farmers. Results of this study confirm that brucellosis is endemic in the country as reported by previous studies (Madzingira et al., 2014; 2015; 2016), at a low level (0.19-0.30%) in the commercial areas and point to a higher prevalence of the disease in the communal areas of Namibia where most cattle (64%) are found (WAHIS, 2018). The low proportion of positive reactors (0.19-0.30%) determined in the commercial sectors (beef, dairy, export) could be an endorsement of the effectiveness of measures applied against brucellosis in these areas. These measures as prescribed by the Animal Health Regulations (2018) of Namibia include the compulsory vaccination of all heifers of 3-8 months of age; annual testing of milk producing animals; quarantine measures and the testing, identification, and disposal of reactor animals. However, future studies based on planned sampling are required to check whether the positive reactors from the commercial sector were false positive reactors from persistent *Brucella abortus* S19 vaccine induced antibodies which may last for up to 4.5 years and interfere with serological reactions (Dorneles et al., 2015; Simpson et al., 2018) or cross reactions with antibodies produced by other organisms with smooth lipopolysaccharides such as *Yersinia enterocolitica* and *Salmonella* (Radostits et al., 2000).

The proportion of positive dairy (0.19%) and beef cattle (beef = 0.30%, export = 0.33%) was low and comparable to previous observations of 0-1.94% in commercial beef cattle in Namibia (Magwedere et al., 2011) and 0.3% reported in dairy cattle in

South Africa (Godfroid et al., 2019). However, it was lower than the proportion of reactors (0.70-5.5%) reported by several studies on large scale commercial dairy farms in Africa (Madzima, 1987; Tesfaye et al., 2011; Tschopp et al., 2013; Asmare et al., 2013; Sacchia et al., 2013; Abdullah et al., 2014; Asgedom et al., 2016; Geresu and Kassa, 2016). Thus, our results when compared with elsewhere in Africa, provide support to the effectiveness of brucellosis control measures on commercial beef and dairy farms in the country. Whereas routine testing for brucellosis in beef cattle is voluntary, annual testing of dairy cattle and the slaughter of positive reactors is compulsory and is enforced by the state.

A low level of reactors (0.33%) in beef cattle (steers and heifers) intended for export was determined. Due to the compulsory requirement for the vaccination of heifers of 3-8 months of age using *Brucella abortus* strain 19 (S19) in Namibia, the contribution of vaccine induced and maternal antibodies to the observed proportion of positive reactors cannot be excluded. Further, it is speculated that most of heifers and steers that were tested, may not have had exposure to the pathogen or had not seroconverted as frequently occurs in persistently infected animals (La Praik et al., 1975; Wilesmith, 1978; Dolan, 1980; Nicoletti, 1980; La Praik and Moffat, 1982; Catlin and Sheehan, 1986; FAO/WHO, 1986; Walker, 1999). Positive reactors were excluded from export and measures were taken against the herd as prescribed in the Animal Health Regulations (2018). Among the imported cattle, no positive reactors were recorded. Therefore, the risk of introducing *Brucella* into the country was considered as minimal during the study period.

Our study confirms that the level of brucellosis infection in the communal areas (10.27%) was higher than in the commercial areas (0.24%) ($p < 0.05$). An overall finding that communal cattle and cattle farms are 48 and 10 times more likely to be positive for brucellosis respectively, should be enough to motivate for more focused control measures for brucellosis in these high-risk locations to prevent public health effects and production losses due to infertility, abortions, and neonatal mortalities. Similar high proportions of positive reactors (15.6%) have been reported in the communal areas of Kwa-Zulu Natal province in South Africa (Hesterberg et al., 2008) and from surveys in traditionally managed systems (16%) in sub-Saharan Africa

(McDermott and Arimi, 2002; Mangen et al., 2002; Mohan et al., 1996; Madsen, 1989; Muma et al., 2013). Free movement and intermingling of differently owned herds occur regularly in the communal grazing areas and at watering points especially during the dry season, facilitating the transmission of this contagious disease, hence the tendency to have more positive reactors (Madzima, 1987; Musa et al., 1990; Muma et al., 2007; Mekonnen et al., 2010; Makita et al., 2011; Mai et al., 2012). Furthermore, because bulls are shared among communal herds, venereal transmission of *Brucella* infection is possible (Bercovich, 1998). Commercial farms are fenced and as a result, the mixing of different herds is precluded.

It is an established fact that vaccination against *Brucella* infection is compulsory in the country, but vaccination of cattle is low or absent in the communal areas due to the lack of awareness, vaccine costs and lack of enforcement; hence the naivety of communal cattle and associated increased risk of production losses (abortions and infertility). It is therefore unlikely that the level of seropositivity observed in the communal areas was confounded by vaccine-induced antibodies. The relatively high level of *Brucella* infection in cattle herds in the communal areas, coupled with limited veterinary services, often results in farmers assisting cows at calving, thus exposing themselves to possible infection. A call for robust surveillance and vaccination campaigns in the communal areas is put forward to decrease the incidence of positive reactors.

A decline in the trend of positive reactors per year that was apparent in the communal areas from 2013 to 2018 is encouraging, as it may reflect improved disease control arising from improvements in the accessibility of veterinary services due to an aggressive deployment of veterinary personnel that occurred in the past few years. This increased veterinary deployment may also have resulted in increased farmer awareness of the disease. In the commercial areas, the annual proportion of reactor cattle remained relatively constant at <0.63% except in 2009 (1.37%), indicating a low level of *Brucella* infection.

In this study, serological testing was based on the RBT and CFT in series, with the former as a screening test and the later as a confirmatory test. The RBT was used

as a screening test because of its relatively high sensitivity (78-100%) (Bercovich, 1998; Díaz-Aparicio et al., 1994), while the CFT was preferred as a confirmatory test because it is a highly specific test (98%) (Bercovich, 1998). The use of both tests is also recommended by the OIE for international trade purposes (OIE, 2004). The RBT was had more false positives in the commercial areas (50.43%) than in the communal areas (17.31%). More than likely, the false positives that were identified by the CFT arose from the widespread use of *B. abortus* S19 vaccine in heifers especially those above eight months of age (Ndazigaruye et al., 2018) or to antibodies elicited by other organisms with antigenic epitopes that are similar to *Brucella* such as *Escherichia, Yersinia* and *Vibrio* (Mainar-Jaime et al., 2005). Culture and isolation, the gold standard for *Brucella* diagnosis, is not yet implemented in Namibia, but conventional *Brucella* PCR, an extremely sensitive assay, has recently been adopted.

A link between history of abortions and seropositivity has been established elsewhere (Matope et al., 2011; Alhaji et al., 2016; Tasiame et al., 2016). In this study, there was no apparent link between the 252 abortion-linked sera from commercial farming and seropositivity, as has been documented by a previous study in dairy cattle in Rwanda (Ndazigaruye et al., 2018). Therefore, other causes of abortions such as *Chlamydophila abortus, Campylobacter fetus* and *Neospora caninum*, need to be investigated in these cattle herds. However, a proportion of the abortion-linked sample (12.65%) tested positive, confirming a link between abortions and seropositivity as has been reported by other studies (McDermott and Arimi, 2002; Schelling et al., 2003; Ibrahim et al., 2010; Boukary et al., 2013). This proportion was within the range of 0.17-16.2% reported for Africa (Ntirandekura et al., 2018), but greater than the 8.3% reported in Zimbabwe under similar farm settings (Gomo et al., 2012). Other clinical signs consistent with brucellosis such as orchitis, stillbirths, retained placentas and hygromas were also recorded as reasons for testing cattle for brucellosis in both production sectors.

Location-wise, *Brucella* antibodies were detected on farms in 12 out of the 14 administrative regions of country, confirming the widespread spatial distribution of low-level brucellosis in Namibia, although the burden weighed heavily against the

communal farm settings. Perhaps, the differing cattle management systems per region and agro-ecological conditions influenced the observed levels of brucellosis detection (Madzima, 1987) as evidenced by the absence of positive reactor herds in the dry, hot regions of Erongo and Kunene.

5.6. Conclusion and recommendations

In this study, recommended serological tests (RBT and CFT) were used to evaluate the sero-epidemiology of brucellosis in Namibia using a robust sample size from passive and active surveillance of brucellosis (clinical cases, export, and dairy testing) over a 15-year period. The study revealed that bovine brucellosis is endemic in Namibia at a low proportion primarily in communal cattle herds. Due to the low level of positive reactors, the risk of importing or exporting brucellosis through cattle was inferred to be minimal. Cases of abortions from the communal areas were more likely to test positive for brucellosis than those from commercial areas. Despite the low proportion of infected bovines, the disease remains a public health risk and a potential cause of production losses in cattle especially in the communal areas where vaccination is limited. Since vaccination has been shown to be effective in reducing the level of infection, its widespread application is encouraged in the communal areas, and this should be backed up by planned routine serological surveillance in the country, especially in the communal areas. Interpretation of the findings of this study should be made with caution because it includes data from passive surveillance which may not be representative of the true picture.

5.7. References

ABDULLAH, A.H., BASHIR, U.M., RAFIQUL, I.M., CHO, H-S. & MUKTER, H.M. 2014. Serological prevalence of brucellosis of cattle in selected dairy farms in Bangladesh. *Korean Journal of Veterinary Research*, 54, 239–243. DOI: 10.14405/kjvr.2014.54.4.239.

AHR, 2018. Animal Health Regulations of the Animal Health Act 1 of 2011 (Government Notice 358 of 2018). https://laws.parliament.na/cms_documents/animal-health-act-1-of-2011---regulations-2018-358-d37ff9f7a0.pdf. *Accessed May 25, 2019*.

AKAKPO, A.J., TÊKO-AGBO, A. & KONÉ, P. 2009. The impact of brucellosis on the economy and public health in Africa. http://www.oie.int/fileadmin/Home/eng/Publications_%26_Documentation/docs/pdf/TT/2009_085-098_Akakpo_A.pdf. *Accessed March 17, 2019*.

ALHAJI, N.B., WUNGAK, Y.S. & BERTU, W.J. 2016. Serological survey of bovine brucellosis in Fulani nomadic cattle breeds (*Bos indicus*) of North-central Nigeria: potential risk factors and zoonotic implications. *Acta Tropica*, 153, 28–35. DOI: 10.1016/j.actatropica.2015.10.003.

ALTON, G. 1991. *Brucella melitensis*. In: J.F. FRANK (eds), Networking in Brucellosis Research, (United Nations University Press), 205–216.

ASGEDOM, H., DAMENA, D. & DUGUMA, R. 2016. Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district, Ethiopia, *Springerplus*, 5, 851. DOI: 10.1186/s40064-016-2547-0.

ASMARE, K., SIBHAT, B., MOLLA, W., AYELET, G., SHIFERAW, J., MARTIN, A.D., SKJERVE, E. & GODFROID, J. 2013. The status of bovine brucellosis in Ethiopia with special emphasis on exotic and cross bred cattle in dairy and breeding farms. *Acta Tropica*, 126, 186-192. DOI: 10.1016/j.actatropica.2013.02.015.

BERCOVICH, Z. 1998. Maintenance of *Brucella abortus* free herds: a review with emphasis on epidemiology and the problems of diagnosing brucellosis in areas of low prevalence. *Veterinary Quarterly*, 20, 81-88. DOI: 10.1080/01652176.1998.9694845.

BOUKARY, A.R., SAEGERMAN, C., ABATIH, E., FRETIN, D., ALAMBE´DJI BADA,

R., DE DEKEN, R., HAROUNA, H.A., YENIKOYE, A. & THYS, E. 2013. Seroprevalence and potential risk factors for *Brucella* spp. infection in traditional cattle, sheep and goats reared in urban, peri-urban, and rural areas of Niger. *PLoS One*, 8, e83175. DOI: 10.1371/journal.pone.0083175.

CADMUS, S.I.B., ADESOKAN, H.K. AND STACK, J., 2008. The use of the milk ring test and Rose Bengal test in brucellosis control and eradication in Nigeria, *Journal of the South African Veterinary Association*, 79, 113–115. DOI: 10.4102/jsava.v79i3.256.

CADMUS, S.I.B., IJABONE, I.F., OPUTA, H.E., ADESOKAN, H.K. & STACK, J.A. 2006. Serological survey of brucellosis in livestock animal and workers in Ibadan Nigeria. *African Journal of Biomedical Research*, 9, 163-168.

CAMPO, L.R., TAMAYO, R. & CAMPO, C.H. 1987. Embryo transfer from brucellosis-positive donors: a field trial. *Theriogenology*, 27, 221.

CATLIN, J.E. & SHEEHAN, E.J. 1986. Transmission of bovine brucellosis from dam to offspring. *Journal of the American Veterinary Medical Association*, 188, 867–869.

CHIMANA, H.M., MUMA, J.B., SAMUI, K.L., HANGOMBE, B.M., MUNYEME, M., MATOPE, G., PHIRI, A.M., GODFROID, J., SKJERVE, E. & TRYLAND, M.A. 2010. Comparative study of seroprevalence of brucellosis in commercial and small-scale mixed dairy-beef cattle enterprises of Lusaka province and Chibombo district, Zambia. *Tropical Animal Health and Production*, 42, 1541-1545. DOI: 10.1007/s11250-010-9604-4.

CORBEL, M.J. 1997. Brucellosis: an overview. *Emerging Infectious Diseases*, 3, 213.

DÍAZ-APARICIO, E., MARÍN, C., ALONSO-URMENETA, B., ARAGÓN, V., PÉREZ-ORTIZ, S., PARDO, M. & MORIYÓN, I. 1994. Evaluation of serological tests for diagnosis of *Brucella melitensis* infection of goats. *Journal of Clinical Microbiology*, 32, 1159-1165. DOI:10.1128/jcm.32.5.1159-1165.1994.

DOLAN, L.A. 1980. Latent carriers of brucellosis. *Veterinary Record*, 106, 241-243.

DORNELES, E.M.S., SRIRANGANATHAN, N. & LAGE, A.P. 2015. Recent advances in *Brucella abortus* vaccines. *Veterinary Research*, 46, 76. DOI: 10.1186/s13567-015-0199-7.

DVS. 1987. Directorate of Veterinary Services Annual Report. South West Africa.

EC. 2001. Brucellosis in sheep and goats *(Brucella melitensis).* https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out59_en.pdf. *Accessed September 23, 2018*.

FAO/WHO, 1986. Joint FAO/WHO Expert Committee on Brucellosis. World Health Organization Technical Report Series, 740, 1-132. https://apps.who.int/iris/handle/10665/40202. Accessed June 18, 2019.

FÈVRE, E., DE GLANVILLE, W., THOMAS, L., COOK, E., KARIUKI, S. & WAMAE, C. 2017. An integrated study of human and animal infectious disease in the Lake Victoria crescent small-holder crop-livestock production system, Kenya. *BMC Infectious Diseases*, 17, 457. DOI: 10.1186/s12879-017-2559-6.

GERESU, M.A. & KASSA, G.M. 2016. A review on diagnostic methods of brucellosis. *Journal of Veterinary Science and Technology*, 7, 323. DOI: 10.4172/2157-7579.1000323.

GODFROID, J. 2002. Brucellosis in wildlife. *Revue Scientifique et Technique- Office International des Épizooties*, 21, 277-286. DOI: 10.20506/rst.21.2.1333.

GODFROID, J., BOSMAN, P.P., HERR, S. & BISHOP, G.C. 2019. Bovine brucellosis. In: J.A.W. COETZER, G.R. THOMSON, N.J. MACLACHLAN & M.L. PENRITH (eds), Infectious diseases of livestock, Part 3. https://anipedia.org/resources/bovine-brucellosis/922. *Accessed May 31, 2019*.

GOMO, C., DE GARINE-WICHATITSKY, M., CARON, A. & PFUKENYI, D. 2012. Survey of brucellosis at the wildlife livestock interface on the Zimbabwean side of the Greater Limpopo Transfrontier Conservation Area. *Tropical Animal Health and Production*, 44, 77-85. DOI: 10.1007/s11250-011-9890-5.

HESTERBERG, U.W., BAGNALL, R., PERRETT, K., BOSCH, B., HORNER, R. & GUMMOW, B.A. 2008. Serological prevalence survey of *Brucella abortus* in cattle of rural communities in the province of KwaZulu-Natal, South Africa. *Journal of the South African Veterinary Association*, 79, 15–18. DOI: 10.4102/jsava.v79i1.234.

HOTEZ, P.J. & GURWITH, M. 2011. Europe's neglected infections of poverty. *International Journal of Infectious Diseases*, 15, 611–619. DOI: 10.1016/j.ijid.2011.05.006.

IBRAHIM, N., BELIHU, K., LOBAGO, F. & BEKENA, M. 2010. Sero-prevalence of

bovine brucellosis and risk factors in Jimma zone of Oromia Region, South-western Ethiopia. *Tropical Animal Health and Production*, 42, 35–40. DOI: 10.1007/s11250-009-9382-z.

Info-Namibia. 2019. Namibia-General information. https://www.info-namibia.com/info/general-information. *Accessed January 15, 2019*.

LA PRAIK, R.D., BROWN, D.D., MANN, H. & BRAND, T. 1975. Brucellosis: a study of five calves from reactor dams. *Veterinary Record*, 97, 52–54. DOI: 10.1136/vr.97.3.52.

LA PRAIK, R.D. & MOFFAT, R. 1982. Latent bovine brucellosis. *Veterinary Record*, 111, 578–579. DOI: 10.1136/vr.111.25-26.578.

LUCERO, N.E., AYALA, S.M. & ESCOBAR, G.I. 2008. *Brucella* isolated in humans and animals in Latin America from 1968 to 2006. *Epidemiology and Infection*, 136, 496-503. DOI: 10.1017/S0950268807008795.

MADSEN, M. 1989. The current status of brucellosis in Zimbabwe. *Zimbabwe Veterinary Journal*, 20, 133-145.

MADZIMA, W.M. 1987. Zimbabwe. Bovine brucellosis and brucellosis of small ruminants: diagnosis, control and vaccination. *Revue Scientifique et Technique- Office International des Épizooties*, 6, 80–82.

MADZINGIRA, O. & MCCRINDLE, C. 2014. Prevalence of *Brucella* antibodies in sheep and springbok (*Antidorcas marsupialis*) reared together in the Karas region, Namibia. *Bulletin of Animal Health and Production in Africa*, 62, 299-306.

MADZINGIRA, O. & MCCRINDLE, C. 2015. Retrospective analysis of the prevalence of *Brucella* antibodies in sheep in the Karas Region of Namibia. *Tropical Animal Health and Production*, 47, 1117. DOI: 10.1007/s11250-015-0838-z.

MADZINGIRA, O. & MCCRINDLE, C. 2016. A questionnaire survey of risk factors of brucellosis on mixed sheep and springbok (*Antidorcas marsupialis*) farms. *International Science and Technology Journal of Namibia*, 8, 43-49.

MADZINGIRA, O. & SEZUNI, M.P. 2017. Serological prevalence and public health significance of brucellosis on a dairy farm in Namibia from 2011-2014. *BMC Research Notes*, 10, 620. DOI: 10.1186/s13104-017-2933-x.

MAGWEDERE, K., BISHI, A., TJIPURA-ZAIRE, G., EBERLE, G., HEMBERGER, Y.,

HOFFMAN, L.C. & DZIVA, F. 2011. Brucellae through the food chain: the role of sheep, goats and springbok (*Antidorcus marsupialis*) as sources of human infection in Namibia. *Journal of the South African Veterinary Association*, 82, 205-212. DOI: 10.4102/jsava.v82i4.75.

MAI, H.M., IRONS, P.C., KABIR, J. & THOMPSON, P.N. 2012. A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. *BMC Veterinary Research*, 8, 144-158. DOI: 10.1186/1746-6148-8-144.

MAINAR-JAIME, R. C., MUÑOZ, P.M., DE MIGUEL, M.J., GRILLÓ, M.J., MARÍN, C.M., MORIYÓN, I. & BLASCO, J.M. 2005. Specificity dependence between serological tests for diagnosing bovine brucellosis in Brucella-free farms showing false positive serological reactions due to Yersinia enterocolitica O:9. *The Canadian Veterinary Journal = La revue veterinaire canadienne*, *46*(10), 913–916.

MAKITA, K., FE`VRE, M.E., WAISWA, C., EISLER, M., THRUSFIELD, M. & WELLBURN, S.C. 2011. Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. *BMC Veterinary Research*, 7, 60. DOI: 10.1186/1746-6148-7-60.

MANGEN, M., OTTE, J., PFEIFFER, D. & CHILONDA P. 2002. Bovine brucellosis in sub-saharan Africa: estimation of seroprevalence and impact on meat and milk offtake potential. Livestock Policy Discussion Paper No. 8, (Food and Agriculture Organisation: Rome). http://www.fao.org/3/a-ag274e.pdf. *Accessed June 21, 2018*.

MATOPE, G., BHEBHE, E., MUMA, J.B., LUND, A. & SKJERVE, E. 2010. Herd-level factors for *Brucella* seropositivity in cattle reared in small holder dairy farms of Zimbabwe. *Preventive Veterinary Medicine*, 94, 213–221. DOI: 10.1016/j.prevetmed.2010.01.003.

MATOPE, G., BHEBHE, E., MUMA, J.B., OLOYA, J., MADEKUROZWA, R.L., LUND, A. & SKJERVE, E. 2011. Seroprevalence of brucellosis and its risk factors in cattle from smallholder dairy farms in Zimbabwe. *Tropical Animal Health and Production*, 43, 975-979. DOI: 10.1007/s11250-011-9794-4.

MCDERMOTT, J. & ARIMI, S. 2002. Brucellosis in sub-Saharan Africa:

epidemiology, control and impact. *Veterinary Microbiology*, 90, 111-134. DOI: 10.1016/s0378-1135(02)00249-3.

MCDERMOTT, J., GRACE, D. & ZINSSTAG, J. 2013. Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique-Office International des Épizooties*, 32, 249–261. DOI: 10.20506/rst.32.1.2197.

MEKONNEN, H., KALAYOU, S. & KYULE, M. 2010. Serological survey of bovine brucellosis in Barka and Arado breeds (*Bos indicus*) of Western Tigray, Ethiopia. *Preventive Veterinary Medicine*, 94, 28–35. DOI: 10.1016/j.prevetmed.2009.12.001.

MOHAN, K., MAKAYA, P.V., MUVAVARIRWA, P., MATOPE, G., MAHEMBE, E. & PAWANDIWA, A. 1996. Brucellosis surveillance and control in Zimbabwe: bacteriological and serological investigation in dairy herds. *Onderstepoort Journal of Veterinary Research*, 63, 47-51.

MUMA, J.B., GODFROID, J., SAMUI, K.L. & SKJERVE, E. 2007. The role of *Brucella* infection in abortions among traditional cattle reared in proximity to wildlife on the Kafue flats of Zambia. *Revue Scientifique et Technique- Office International des Épizooties*, 26:721–730.

MUMA, J.B., SAMUI, K.L., OLOYA, J., MUNYEME, M. & SKJERVE, E. 2007. Risk factors for brucellosis in indigenous cattle reared in livestock–wildlife interface areas of Zambia. *Preventive Veterinary Medicine*, 80, 306-317. DOI: 10.1016/j.prevetmed.2007.03.003.

MUMA, J.B., SYAKALIMA, M., MUNYEME, M., ZULU, V.C., SIMUUNZA, M. & KURATA, M. 2013. Bovine tuberculosis and brucellosis in traditionally managed livestock in selected districts of southern province of Zambia. *Veterinary Medicine International*. DOI: 10.1155/2013/730367.

MUSA, M.T., JAHANS, K.L. & FADALLA, M.E. 1990. Clinical manifestation of brucellosis in cattle of the southern Dafur province, western Sudan. *Journal of Comparative Pathology*, 103, 95–99. DOI: 10.1016/s0021-9975(08)80139-9.

MUSHENDAMI, P., BIWA, B. & GAOMAB II, M. 2008. Unleashing the potential of the agricultural sector in Namibia. https://www.bon.com.na/CMSTemplates/Bon/Files/bon.com.na/a2/a2d7d502-f176-432e-9fdd-3de89d3dbb92.pdf. *Accessed April 12 2019*.

NDAZIGARUYE, G., MUSHONGA, B., KANDIWA, E., SAMKANGE, A. & SEGWAGWE, B. E. 2018. Prevalence and risk factors for brucellosis seropositivity in cattle in Nyagatare District, Eastern Province, Rwanda. *Journal of the South African Veterinary Association*, 89, 1625. DOI: 10.4102/jsava.v89i0.1625.

NICOLETTI, P. 1980. The epidemiology of bovine brucellosis. *Advances in Veterinary Science and Comparative Medicine*, 24, 69–98.

NTIRANDEKURA, J-B., MATEMBA, L.E., KIMERA, S.I., MUMA, J.B. & KARIMURIBO, E.D. 2018. Association of brucellosis with abortion prevalence in humans and animals in Africa: a review. *African Journal of Reproductive Health*, 22, 120-136. DOI: 10.29063/ajrh2018/v22i3.13.

OIE. 2004. Office International des Épizooties. Bovine brucellosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris: OIE, 2004:409–438.

PAPPAS, G. 2010. The changing *Brucella* ecology: novel reservoirs, new threats. *International Journal of Antimicrobial Agents*, 36(Suppl 1), S8-S11. DOI: 10.1016/j.ijantimicag.2010.06.013.

RADOSTITS, O.M., GAY, C.C., BLOOD, C.D. & HINCHCLIFF, K.W. 2000. Veterinary Medicine. A textbook of the disease of cattle, sheep, pigs, goats and horses, 9th edition, (W.B. Saunders Company Ltd : New York).

REICZIGEL, J., FÖLDI, J. & ÓZSVÁRI, L. 2010. Exact confidence limits for prevalence of a disease with an imperfect diagnostic test. *Epidemiology and Infection*, 138, 1674–1678. DOI: 10.1017/S0950268810000385.

SACCHIA, M., DI PROVVIDO, A., IPPOLITI, C., KEFLE, U., SEBHATU, T.T.D., ANGELO, A. & DE MASSIS, F. 2013. Prevalence of brucellosis in dairy farming regions of Eritrea. *Onderstepoort Journal of Veterinary Research*, 80, 448. DOI: 10.4102/ojvr.v80i1.448.

SCHELLING, E., DIGUIMBAYE, C., DAOUD, S., NICOLETTI, J., BOERLIN, P., TANNER, M. & ZINSSTAG, J. 2003. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine*, 61, 279–293. DOI: 10.1016/j.prevetmed.2003.08.004.

SIMPSON, G.J.G., MARCOTTY, T., ROUILLE, E., CHILUNDO, A., LETTESON, J-J.

& GODFROID, J. 2018. Immunological response to *Brucella abortus* strain 19 vaccination of cattle in a communal area in South Africa. *Journal of the South African Veterinary Association*, 89, 1–7. DOI: 10.4102/jsava.v89i0.1527.

STEHN, H. 2008. Large stock management. http://www.agrinamibia.com.na/wp-content/uploads/2018/02/3-Large-Stock-Manual.pdf. A*ccessed December 12, 2018*.

TASIAME, W., EMIKPE, B., FOLITSE, R.D., FOFIE, C.O., BURIMUAH, V., JOHNSON, S., AWUNI, J.A., AFARI, E., YEBUAH, N. & WURAPA, F. 2016. The prevalence of brucellosis in cattle and their handlers in North Tongu District, Volta Region, Ghana, African *Journal of Infectious Diseases*, 10, 111–117. DOI: 10.21010/ajid.v10i2.6.

TESFAYE, G., TSEGAYE, W., CHANIE, M. & ABINET, F. 2011. Seroprevalence and associated risk factors of bovine brucellosis in Addis Ababa dairy farms, *Tropical Animal Health and Production*, 43, 1001–1005. DOI: 10.1007/s11250-011-9798-0.

TSCHOPP, R., ABERA, B., SOUROU, S.Y., GUERNE-BLEICH, E., ASEFFA, A., WUBETE, A., ZINSSTAG, J. & YOUNG, D. 2013. Bovine tuberculosis and brucellosis prevalence in cattle from selected milk cooperatives in Arsi zone, Oromia region, Ethiopia. *BMC Veterinary Research*, 9, 163. DOI: 10.1186/1746-6148-9-163.

WAHIS. 2018. World Animal Health Information System. http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalpopulation. *Accessed June 28, 2019*.

WALKER, R.L. 1999. Brucella. In: D.C. HIRSH and Y.C. ZEE (eds), Veterinary Microbiology, (Blackwell Science Inc.: USA), 196-203.

WHO. 1965. World Health Organization Regional Office for South-East Asia, Seventeenth Annual Report of The Regional Director to the Regional Committee for South-East Asia, New Delhi: http://www.who.int/iris/handle/10665/126772 *Accessed March 12, 2020*.

WHO. 2005. Brucellosis in humans and animals. WHO guidance. In: L. HEYMANN (ed), Control of communicable diseases manual: an official report of the American Public Health Association, (World Health Organization/America Public Health Association, Washington DC).

WHO. 2006. Brucellosis in humans and animals. https://apps.who.int/iris/handle/10665/43597. Accessed July 28, 2018.

WILESMITH, J.W. 1978. The persistence of *Brucella abortus* infection in calves: a retrospective study of heavily infected herds. *Veterinary Record*, 103, 149–153. DOI: 10.1136/vr.103.8.149.

YOUNG, E.H. 1995. An overview of human brucellosis. *Clinical Infectious Diseases*, 21, 283-289. DOI: 10.1093/clinids/21.2.283.

Chapter 6: Seroprevalence and molecular confirmation of *Brucella abortus* from bovine tissues at an abattoir in Namibia

Oscar Madzingira[1,2], Siegfrid Khaiseb[3], Georgina Tjipura-Zaire[3], Gottlieb Aikukutu[3], Fidelis Kandongo[3], Francis B. Kolo[2], Henriette van Heerden[2]

[1]School of Veterinary Medicine, Faculty of Agriculture and Natural Resources, University of Namibia, Namibia
[2]Department of Veterinary Tropical Diseases, Room 2-11, Paraclinical Building University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa.
[3]Directorate of Veterinary Services, Ministry of Agriculture, Water and Land Reform, Government Office Park, Windhoek

6.1. Abstract

Brucellosis is a worldwide zoonosis of significant public and veterinary health importance that is endemic in Namibia. Data on the prevalence of the disease and *Brucella* spp. infecting cattle is essential for the implementation of appropriate preventive and control measures. Therefore, the aim of this study was to estimate seroprevalence of brucellosis; to identify *Brucella* species in cattle tissues at a major beef abattoir in Namibia using the genus specific 16-23S rRNA interspacer (ITS) PCR and to characterize *Brucella* species from cultures using the species-specific AMOS-PCR. Between December 2018 and May 2019, a serosurvey was conducted in cattle (n = 304) at the abattoir using the Rose Bengal test (RBT) as a screening assay and the complement fixation test (CFT) as a confirmatory test. Concurrently, an identical number of pooled lymph nodes and spleen were collected from the same animals. However, only 200 sets of tissues (lymph nodes and spleen) from randomly selected seronegative cattle were subjected to conventional ITS-PCR. Seven pairs of tissues (pooled lymph nodes and spleen) from seropositive cattle (n = 7) were also subjected to conventional ITS-PCR. Homogenates of these tissues were cultured and isolates characterized using AMOS-PCR. Prevalence of antibodies against *Brucella* in slaughtered cattle was 2.3% (7/304) based on the RBT and 1.6% (5/304) after confirmation with the CFT. Herd prevalence was estimated at 9.6% (5/52).

Brucella spp. DNA was identified in tissues [(lymph nodes (85.7%, 6/7) and spleen (85.7%, 6/7)] from four RBT and CFT seropositive cattle and two RBT positive cattle but was not detected in tissues from one RBT and CFT positive animal. Cultures of lymph nodes (51.4%, 4/7) and spleen (85.7%, 6/7) from seropositive cattle (RBT or CFT) yielded *Brucella* growth and were identified as *Brucella abortus* using AMOS-PCR. *Brucella* spp. DNA was not detected in spleen or lymph nodes from selected seronegative cattle (n = 200). Although abattoir prevalence was low, the detection of seropositive cattle and *B. abortus* indicates a risk for *Brucella* infection in abattoir workers and potential production losses on the farms of origin. Therefore, control of brucellosis on farms and the promotion of brucellosis awareness, use of adequate protective gear and safe meat handling practices at the abattoir are recommended to prevent infection among abattoir workers.

6.2. Introduction

Brucellosis is a zoonosis caused by *Brucella* bacteria, a genus comprising of highly homologous species (> 90% DNA homology) (Halling et al., 2005). *Brucella abortus, B. melitensis* and *B. suis* cause the greatest impact on animal production (OIE, 2018). *Brucella abortus* is the most common cause of bovine brucellosis around the world, with huge financial losses reported in several countries (Moreno, 2002; Godfroid et al., 2004). In southern Africa*, B. abortus* biovar 1 is the predominant isolate in cattle (Bishop et al., 1994; Mohan et al., 1996; Kolo et al., 2019). In rare cases, *B. melitensis* (Verger, 1985; Jimenez De Bagues et al., 1991; Corbel, 1997; Godfroid et al., 2013a; Kolo et al., 2019) and *B. suis* biovars (Alton et al., 1988; Corbel, 1997; Ledwaba et al., 2019) have been isolated from cattle.

Epidemiological reports from around the world showed that brucellosis is a neglected (Pappas, 2010) and thus a re-emerging disease among animal and human populations (WHO, 2011), with an estimated 5 000 000 to 12 500 000 human cases reported annually (WHO, 1997; Godfroid et al., 2013b; Berger, 2016). Brucellosis remains endemic in many developing countries (McDermott and Arimi, 2002), with estimates of prevalence in bovines in Africa ranging from 0-68% (McDermott et al., 2013).

In cattle, *Brucella* infection is transmitted through the ingestion of, contact with or inhalation of contaminated material especially aborted fetuses, vaginal discharges, fetal membranes, milk, feed, or water (Garin-Bastuji et al., 1998; Mangen et al., 2002). It manifests primarily with reproductive problems including late term abortions, retained placenta, stillborn or weak calves, epididymitis, orchitis and infertility (Spickler, 2018). In humans, *Brucella* infection is a food safety and occupational health challenge, with unpasteurized milk and dairy products and contact with infected animal tissues posing the greatest risk for infection (Godfroid et al., 2005; Carvalho Neta et al., 2010). Abattoir workers, cattle herders, veterinarians, dairy workers, livestock farmers, laboratory workers, tanners and hunters are at a greater risk of occupational exposure to *Brucella* infection (EC, 2001; Godfroid, 2002) due to regular contact with animals and animal products. Human brucellosis causes a severe debilitating febrile illness that often results in the loss of productive working hours. The disease manifests non-specific clinical symptoms such as an undulating fever, anorexia, headache, myalgia, back pain, weight loss, chronic fatigue, or polyarthritis (Godfroid, 2002; Carvalho Neta et al., 2010).

Incidence and distribution of human brucellosis typically corresponds with that in the animal population (WHO, 2006). Therefore, the disease situation in cattle can give an indication of prevalence in humans. In 2018, 122 679 cattle were slaughtered at cattle abattoirs in Namibia, which represents about a third of cattle marketed in the country (Meat Board, 2019). Therefore, in addition to herd screening on farms, surveillance at abattoirs can be an invaluable complementary tool for detecting and acting against *Brucella* infected cattle herds and can also be used to assess the risk of occupational exposure in abattoir workers. Control of bovine brucellosis in Namibia is based on measures that are prescribed in the Animal Health regulations (2018). These measures include compulsory vaccination of heifers of 3-8 months with *Brucella* abortus S19 and those older than 8 months of age with RB51, importation of brucellosis-free cattle, identification of positive cases and herds, quarantine procedures and culling of seropositive cases.

Seroprevalence studies have shown that bovine brucellosis is endemic in Namibia with an estimated animal prevalence of 0.49% (Madzingira et al., 2020). Despite the

low brucellosis prevalence, the handling of carcasses, organs and fluids of cattle can expose abattoir workers to *Brucella* infection (Mukhtar, 2010; Swai and Schoonman, 2009; Mirambo et al., 2018), especially as cattle of unknown brucellosis status are slaughtered and abattoir workers often carry injuries on their hands (Banjo et al., 2013). Previous studies in livestock in Namibia (Magwedere et al., 2011; Madzingira and McCrindle, 2014, 2015, 2016; Madzingira and Sezuni, 2017) have been based on serological assays (Rose Bengal and complement fixation tests in series) only. Serology has limited the detection of *Brucella* spp. in cattle in Namibia to genus level. Thus, the species causing infection have not been identified, but presumed to be *B. abortus*. Therefore, the aim of this study was to determine the serological prevalence of bovine brucellosis at a high throughput abattoir and to isolate and identify *Brucella* spp. from tissues and cultures. This information is necessary to improve understanding of the epidemiology of the disease in Namibia and for recommending measures for successful prevention and control of the disease.

6.3. Materials and methods

6.3.1. Study area

Namibia is situated in the South-Western part of Africa at -22°58'1.42"S and 18°29'34.80"E and divided into 14 administrative regions as shown in Figure 6.1. The veterinary cordon fence (VCF) divides the country into the northern communal areas (NCA) and the southern areas that are made up of predominantly commercial livestock farms. The NCA is divided into the FMD protection and infected zones, while areas south of the VCF comprise of the World Organisation for Animal Health (OIE) recognized Foot-and-Mouth disease (FMD)-free areas without vaccination (Figure 6.1). The cattle population in the country is estimated at 2.7 million. Cattle that were slaughtered at the study abattoir originated from farms and a feedlot south of the VCF.

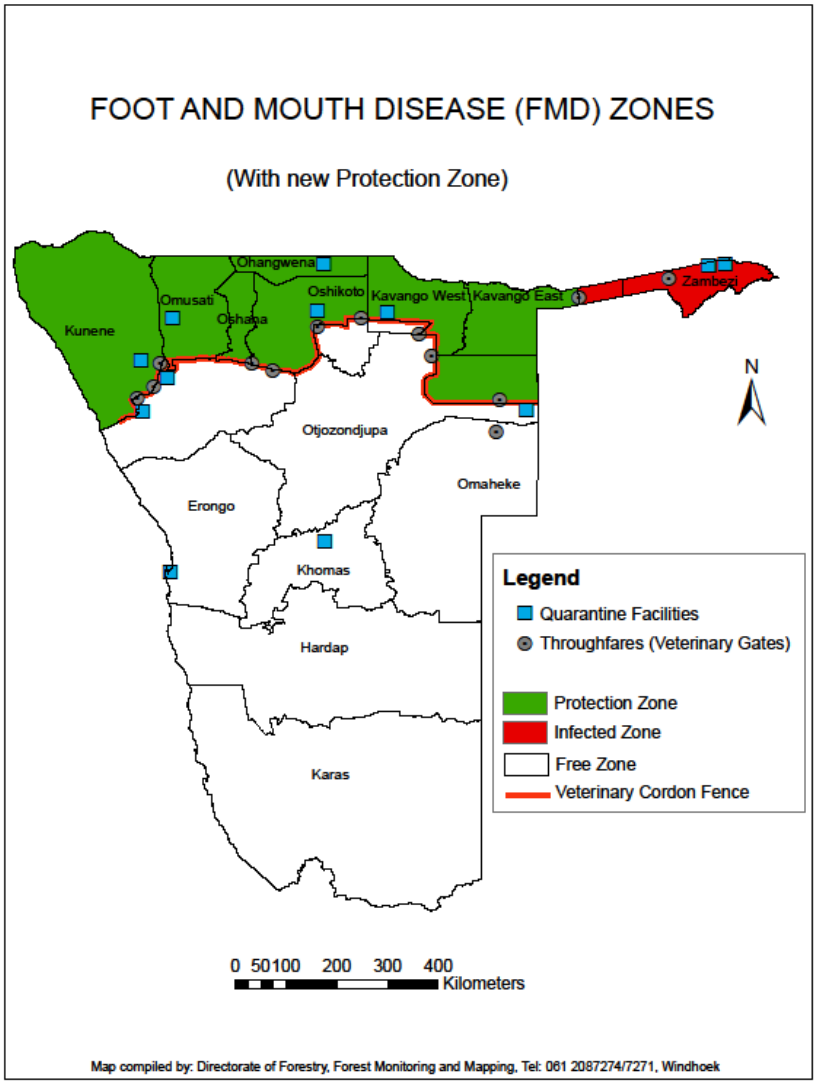


Figure 6.1: Map of Namibia showing the 14 administrative regions and the foot and mouth disease (FMD) zones. The study abattoir was in the Khomas region.

6.3.2. Study abattoir

The abattoir slaughtered cattle of different breeds originating from both commercial and communal farming systems, as well as from auctions and feedlots. With a daily slaughter capacity of 500 cattle, the abattoir slaughtered about half of the annual number of cattle slaughtered at abattoirs in the country (Meat Board, 2019). Ante-mortem and post-mortem inspections, and hygiene procedures were carried out under the supervision of an official veterinarian supported by a team of meat inspectors.

6.3.3. Study design

A cross-sectional study design, using systematic random sampling to select slaughtered cattle for sampling, was used to estimate the prevalence of bovine brucellosis at the abattoir from December 2018 to May 2019. Over the study period, sampling was carried out once a week on a different day of the week. Sampled animals were identified using individual electronic ear tag numbers. Cattle of all ages brought for slaughter at the abattoir were eligible for the study. Age, sex, farm of origin and movement history of each sampled animal were retrieved from slaughter records and from the Namibia Livestock Identification and Traceability System (NamLITS) database through the state veterinarian supervising the establishment.

6.3.4. Sample size

To estimate seroprevalence, a sample size of 304 cattle was determined using the formula $n = 4PQ/L^2$ (Martin et al., 1987), assuming a 5% brucellosis prevalence in the cattle population, a precision of 0.025 (2.5%) and based on a 95% level of confidence.

6.3.5. Sample collection

6.3.5.1. Blood sampling

Blood samples (n = 304) were collected aseptically at the abattoir from severed jugular veins of selected cattle of both sexes. The slaughter procedure was performed following OIE guidelines on animal welfare. For each animal selected for sampling, 5 ml of blood was collected in a sterile plain vacutainer blood tube for serum recovery. The collected samples were identified, securely packed and transported to the Central Veterinary Laboratory (Windhoek), where serum was recovered from clotted blood by centrifugation at 3000 rpm for 5 minutes. Sera were frozen at -20 ^{o}C until testing.

6.3.5.2. Tissue sampling

Pieces of lymph nodes [retropharyngeal, parotid, mandibular, superficial inguinal (in males) and supra-mammary (in females)] and spleen were taken aseptically from the same cattle from which blood was collected and placed in sterile dilution bags.

Lymph node samples from one animal were pooled, while spleen samples were stored separately. Samples were stored at -20 ^{o}C.

6.3.6. Testing of sera

Sera were screened for anti-*Brucella* antibodies using the Rose Bengal test (RBT). Samples testing positive on RBT were subjected to the complement fixation test (CFT) for confirmation. The RBT and CFT were performed as described by the OIE (2018) using standardized antigens (*B. abortus* Weybridge strain 99) for the detection of smooth anti-*Brucella* antibodies. On the RBT, any visible agglutination or clumps were considered as indicative of a positive result (OIE, 2018). CFT results were read after the plates were left to stand for one hour to allow unlysed cells to settle. Titres of 1:8 and above were considered as positive based on the absence of haemolysis. In all cases, positive and negative controls were run with each batch of tests for the purposes of test validation. The RBT has a specificity of 71–80% and a sensitivity of 78–100% (Bercovich, 1998; Díaz-Aparicio et al., 1994), while the CFT has a specificity and a sensitivity of 98% and 81% respectively (Bercovich, 1998).

6.3.7. Isolation and identification of *Brucella* spp.

Bacteriological isolation was performed at the Faculty of Veterinary Science (University of Pretoria, South Africa) in a biosafety level 2+ laboratory on tissues (spleen and lymph nodes, n = 14) obtained from seropositive cattle (n = 7). Homogenates (200 µl) were prepared from each tissue (spleen and lymph nodes) and inoculated onto Farrell's (OIE, 2018) and CITA (Ledwaba et al., 2020) media. The culture plates were incubated at 37°C in the presence of 5% carbon dioxide and observed daily for 14 days for any growth of *Brucella*-like colonies (pinpoint, smooth, translucent, shiny, convex). Typical colonies were presumptively identified by microscopic examination for morphology, size, and staining properties after modified Ziehl-Neelsen staining (OIE, 2018).

6.3.8. Molecular identification of *Brucella* spp. in cattle tissues

Spleen and lymph nodes (n = 207 each) from 207 cattle that comprised five cattle that tested positive on both RBT and CFT; two cattle that were seropositive on RBT only, and 200 randomly selected seronegative cattle (on both RBT and CFT), were

subjected to the *Brucella* genus specific 16S-23S rRNA interspacer region (ITS) conventional PCR. Genomic DNA extraction and purification from tissues was performed following the protocol described in the PureLink® Genomic DNA kit (Life Technologies™). The concentration of extracted DNA was quantified using a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). Genus-specific 16S-23S rRNA interspacer region (ITS) primers were used in a conventional PCR to amplify a 214 bp fragment using the primers ITS66: ACATAGATCGCAGGCCAGTCA and ITS279: AGATACCGACGCAAACGCTAC. The PCR assay was performed as described by Keid et al. (2007). Primers were used at a final concentration of 0.2 µM with 1× DreamTaq Green PCR Master Mix (ThermoFisher Scientific, South Africa) and 2 µl DNA in a 15 µl PCR reaction mixture. The initial PCR assay denaturation was done at 95°C for 3 minutes followed by 35 cycles at 95°C for 1 min, 60°C for 2 min, 72°C for 2 min and finally at 72°C for 5 minutes. *Brucella melitensis* Rev 1 (Onderstepoort Biological Products, South Africa) was used as a positive control and nuclease-free water as a negative control for the PCR assay. Gel electrophoresis of amplicons was performed on a 2% agarose gel stained with ethidium bromide (1.0 g/ml) and the readings were made under UV light.

6.3.9. Molecular characterization of *Brucella* spp. from cultures

The multiplex AMOS-PCR assay was used to identify and differentiate *Brucella* spp. on cultures. The assay was performed as previously described (Bricker and Halling, 1994; Weiner et al., 2011) using DNA extracted from cultures. Four forward primers that are specific to each of the four *Brucella* species under investigation (Table 6.1) were used at a final concentration of 0.1 µM, to which was added 0.2 µM of the reverse primer *IS711* (Table 6.1), 1× MyTaq™ Red PCR Mix (Bioline South Africa) and 2 µl of template DNA in a 25 µl PCR reaction mixture. The PCR assay was performed in the following cycles and conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 1 min, 55.5°C for 2 min, 72°C for 2 min and a final extension step at 72°C for 10 min. Amplicons were analysed by electrophoresis using a 2% agarose gel stained with ethidium bromide that was viewed under UV light. *Brucella abortus* RB51 (Colorado Serum Company, Denver) was used as a positive control, while nuclease free water served as a negative control.

Table 6.1: Oligonucleotide sequences of primers that were used in the AMOS-PCR assay to detect the *Brucella* species and the expected sizes of amplicons.

Name of primer	Sequence (5′-3′)	Size of amplicon (bp)
B. abortus	GAC GAA CGG AAT TTT TCC AAT CCC	498
B. melitensis	AAA TCG CGT CCT TGC TGG TCT GA	731
B. ovis	CGG GTT CTG GCA CCA TCG TCG GG	976
B. suis	GCG CGG TTT TCT GAA GGT GGT TCA	285
IS711	TGC CGA TCA CTT AAG GGC CTT CAT	

6.3.10. Data analyses

Test results were stored in Microsoft Excel® spreadsheet version 2007 (Microsoft Corporation, Redmond, WA). Abattoir or herd prevalence of *Brucella* infection was determined as a percentage of animals or herds tested that were positive on both RBT and CFT positive. The 95% confidence intervals (CI) were estimated considering CFT sensitivity and specificity of 81% and 98% respectively. Proportions of reactors were compared between groups using the z-test. In all cases, $p < 0.05$ was considered significant.

6.3.11. Ethical approval

Authorization to conduct the study was obtained from the abattoir operator. The study protocol was approved by the Chief Veterinary Officer, Ministry of Agriculture, Water and Land Reform (Namibia) (Appendix 11); Director of Animal Health (South Africa) according to Act 35 of 1984 (REF 12/11/1/1/6 (905) (Appendix 8 and Appendix 9); Research Ethics Committee (REC 056-20) (Appendix 6) and Animal Ethics Committee (V055-18) (Appendix 3-5) of the University of Pretoria.

6.4. Results

Between December 2018 and May 2019, lymph nodes and spleen were taken from each of 304 cattle from 52 farms at an abattoir. Most of the cattle sampled (57.9%, n = 176) were female and ≥ 5 years old (45.7%, n = 139), but cattle aged 4 years (n = 40), 3 years (n = 58), 2-2.5 years (n = 40) and < 2 years (n = 27) were also part of the study.

6.4.1. Seroprevalence

Of the 304 sera tested, 7 were positive on RBT, giving an apparent animal prevalence of brucellosis at the abattoir of 2.3% (7/304; 95% CI: 1.1-4.7%). However, after confirmation with the CFT assay, animal brucellosis prevalence was 1.6% (5/304; 95% CI: 0.7-3.8%). Two sera tested positive on RBT, but negative on CFT (titre = 1:4) (Table 6.2). Overall, five cattle herds tested positive for *Brucella* antibodies on the CFT (Table 6.2). Thus, herd prevalence was estimated at 9.6% (5/52; 95% CI: 4.2-20.6%). Of the five animals that tested positive on RBT and CFT, four were males and one was a cow (Table 6.2). Prevalence of anti-*Brucella* antibodies in females (0.6%, 1/176) and males (3.1%, 4/128) was not significantly different ($z = 1.7$, $p = 0.08$). The positive titres were higher in older than in younger animals. Within age categories, prevalence was 5.0% (2/40, 2.0-2.5 years); 2.5% (1/40, 4 years); 2.9% (4/139, ≥ 5 years); 0.0% (0/27, < 2 years) and 0.0% (0/58, 3 years). Of the five CFT-positive cattle, two had a history of movement between farms and through an auction before they were slaughtered at the abattoir (Table 6.2).

6.4.2. Performance of test assays

The proportion of agreement between different assay results is indicated in Table 6.3. Results of ITS-PCR were more agreeable with those of the RBT (85.7%, 6/7) than the CFT (57.1%, 4/7). All tests that were used (RBT, CFT, ITS PCR, culture and isolation and AMOS-PCR) agreed on 57.1% (4/7) of the samples tested. There was a 100% agreement between results of isolation and the AMOS-PCR.

Table 6.2: ITS-PCR and AMOS-PCR screening results of tissues (spleen and lymph nodes) and tissue homogenate cultures from cattle that were positive on RBT (n = 2) and CFT (n = 5).

					***Brucella* spp. DNA (ITS-PCR) (+/-)**		**Culture (+/-)**		**AMOS-PCR on cultures (+/-)**	
Age (years)	**Sex (M/F)**	**History of movement**	**RBT (+/-)**	**CFT titre**	Lymph nodes	Spleen	Lymph nodes	Spleen	Lymph nodes	Spleen
≥5	F	Stayed on one farm throughout	+	1:32 (+)	-	-	-	+	-	+
2-2.5	M	Stayed on one farm throughout	+	1:8 (+)	+	+	+	+	+	+
2-2.5	M	Stayed on two farms and moved through one auction	+	1:8 (+)	+	+	+	+	+	+
4	M	Stayed on one farm throughout	+	1:16 (+)	+	+	+	+	+	+
≥5	M	Moved through two farms and two auctions	+	1:32 (+)	+	+	+	+	+	+
≥5	M	Moved through two farms and two auctions	+	1:4 (-)	+	+	-	+	-	+
≥5	F	Stayed on one farm throughout	+	1:4 (-)	+	+	-	-	-	-

Table 6.3: A comparison of assay performances on samples (n = 7) from seropositive cattle.

Assays	Number positive or negative on both tests	Agreement (%)
RBT and CFT	5	71.4
RBT and ITS-PCR	6	85.7
RBT and Isolation	6	85.7
RBT and AMOS-PCR	6	85.7
CFT and ITS-PCR	4	57.1
CFT and Isolation	6	85.7
CFT and AMOS-PCR	6	85.7
Isolation and AMOS-PCR	7	100.0
Isolation and ITS-PCR	5	71.4

6.4.3. ITS-PCR on tissues

Brucella DNA was detected in both spleen and lymph nodes of 6/7 (85.7%) cattle that were seropositive on RBT, CFT or both (Table 6.2) (Figure 6.2), but not in one animal that was seropositive on both RBT and CFT (Table 6.2) (Figure 6.2). Additionally, spleen and lymph nodes (n = 200 each) that were tested from randomly selected seronegative cattle, also tested negative for *Brucella* DNA. Therefore, the overall detection rate of *Brucella* DNA in tissues was 2.9% (6/207) on ITS-PCR.

6.4.4. Bacterial isolation

Of the lymph nodes (n = 7) and spleen (n = 7) cultured from the seropositive cattle, 57.1% (4/7) and 85.7% (6/7) respectively, yielded *Brucella* isolates from mixed cultures. The 10 isolates had typical phenotypic and morphological characteristics of *Brucella* spp. ITS-PCR confirmed all isolates as *Brucella* spp.

6.4.5. AMOS-PCR results

All isolates from spleen (n = 6) and lymph node (n = 4) homogenate cultures were identified as *B. abortus* by AMOS-PCR (Figure 6.3). *Brucella abortus* was detected more in spleens (85.7%, 6/7) than in lymph nodes (57.1%, 4/7). Results of the RBT, CFT, ITS-PCR, culture and AMOS-PCR agreed in 57.1% (4/7) of the cattle tested (Table 6.2). In one case (14.3%), ITS-PCR did not detect *Brucella* DNA in spleen or lymph nodes, but AMOS-PCR identified *B. abortus* in cultures.

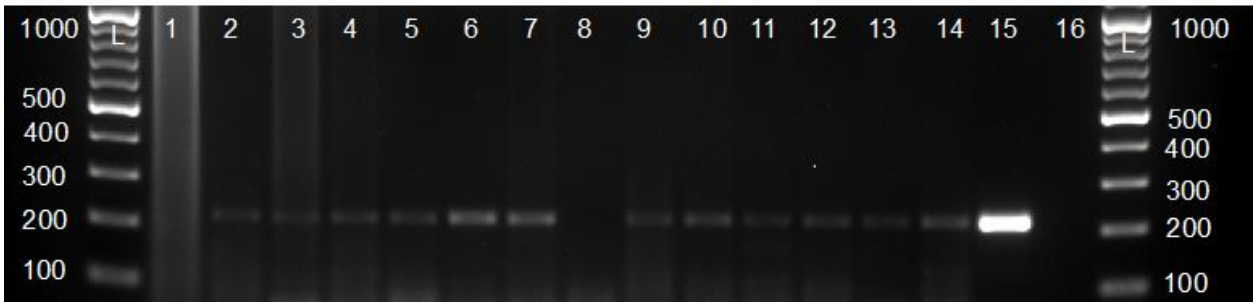


Figure 6.2: Genus-specific 16S-23S rRNA interspacer region (ITS) PCR amplification gel electrophoresis results of tissue samples (lymph nodes and spleen) from seropositive cattle. Lane L (100 bp marker), Lane 1-7 (lymph nodes), Lane 8-14 (spleen), Lane 15 (positive control: *B. melitensis* Rev 1), Lane 16 (negative control: nuclease-free water).

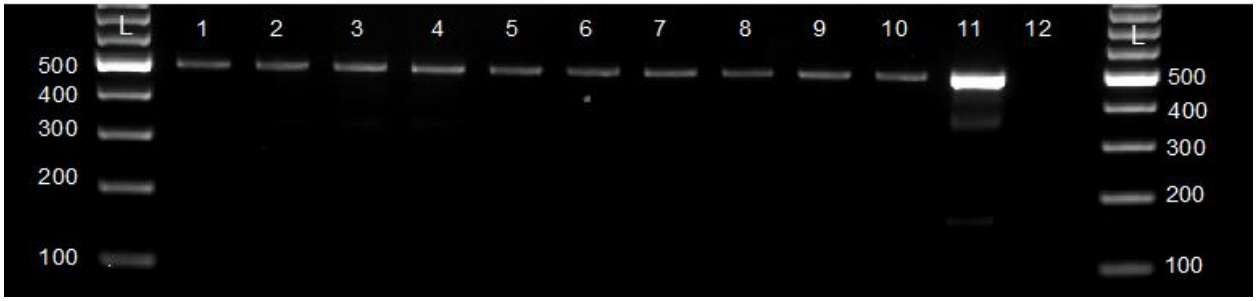


Figure 6.3: AMOS-PCR products from the amplification of the *IS711* gene using *Brucella* species-specific primers. Lane L (100 bp marker), Lane 1-6 (spleen), Lane 7-10 (lymph nodes), Lane 11 (*B. abortus* RB51- positive control), Lane 12 (negative control: nuclease free water).

6.5. Discussion

This study determined seroprevalence and identified *Brucella abortus* in spleen and lymph nodes of seropositive cattle slaughtered at a major cattle abattoir in Namibia using serology, ITS-PCR, culture, and AMOS-PCR. A low abattoir seroprevalence (1.6%) that was determined in this study based on RBT and CFT is confirmation of *Brucella* spp. infection in cattle in Namibia and an indication of low infection rates on the farms of origin. The seroprevalence was within the range of 0.0–2.9% reported for South African (Bishop, 1984; Kolo et al., 2019) and Brazilian abattoirs (Mioni et al., 2018), but higher than the prevalence of up to 0.5% reported by earlier studies in Namibia (DVS, 1987; Madzingira et al., 2020). The slaughter of predominantly

mature cattle may have overestimated the seroprevalence of brucellosis in the current study. Higher abattoir prevalence rates than in the current study have been reported in Nigeria (3.9%) (Akinseye et al., 2016), Tanzania (4.7%) (Luwumba et al., 2019) and several African countries (Shey-Njila et al., 2005; Cadmus et al., 2006; Berhe et al., 2007; Swai and Schoonman, 2009; Mergesa et al., 2011; Cadmus et al., 2013; Awah-Ndukum et al., 2018). The proportion of infected cattle herds (9.6%) at the abattoir was low and similar to a herd prevalence (9.3%) reported previously in Namibia (Madzingira et al., 2020). Results of this study, when compared to previous prevalence studies in Namibia, show that bovine brucellosis prevalence is low and has attained endemic stability in cattle populations.

The positive titre and the number of seropositive cases recorded at the abattoir were higher in older than younger cattle, in agreement with previous studies (Chaka et al., 2018; Selim et al., 2019) and serve to confirm that brucellosis is a disease of sexually mature cattle (Muma et al., 2006; Matope et al., 2011). The higher number of seropositive cases in older than younger animals can be ascribed to a longer exposure time to infection in the herd (Selim et al., 2019), the absence of seroconversion or higher resistance to *Brucella* infection in younger animals (Deka et al., 2018). In contrast to previous studies that found more *Brucella* infected female than male cattle (Kebede et al., 2008; Tolosa et al., 2008; Megersa et al., 2011), the current study found no significant differences in the prevalence between sexes as has also been reported by Shafee et al. (2012). In this study, there was no apparent association between animal movement history and seropositivity, as some seropositive cattle had stayed on one farm throughout their lifetime, while others had been traded between farms and moved through auctions. In earlier studies, Stringer et al. (2008) reported an association between cattle movement history and seropositivity.

Since the vaccination status of cattle in this study was unknown, the contribution of vaccine antibodies to the observed seroprevalence cannot be excluded. However, the fact that four of the five seropositive cattle were male, suggests that these animals had *Brucella* infection (past or present), since male animals are not vaccinated against brucellosis. However, it is possible that farmers may have

vaccinated males out of ignorance. In Namibia, heifers of 3-8 months of age should be vaccinated against brucellosis using S19 and those older than 8 months of age with RB51 as per the Animal Health regulations (2018). This requirement is strictly enforced in the commercial livestock sector, but not in the communal cattle rearing system (Madzingira et al., 2020), both of which supply cattle for slaughter at the study abattoir.

Although the number of samples that were used to compare test agreements were small (n = 7) and included only samples positive on RBT, results indicate that RBT had a higher proportion of agreement with ITS-PCR than CFT. Thus, despite its drawbacks, the RBT is a useful screening test for identifying *Brucella* positive cattle and any negative results on the confirmatory CFT may need to be investigated further using other tests. The identification of *Brucella* DNA in tissues (lymph nodes and spleen) from two RBT positive and CFT negative cattle, reinforces the need to use several tests including ITS-PCR to complement the diagnosis of brucellosis (El-Diasty et al., 2018) in cattle. Under normal circumstances, sera that test positive on RBT and negative on CFT, are considered negative. The discrepancy between the results of the CFT and ITS-PCR may be explained, in part, by the stage of *Brucella* infection at the time of testing. Serological testing of cattle in the early stages of infection using CFT is associated with negative results due to low IgG titres (Taleski, 2010). The absence of *Brucella* DNA in tissues from one RBT and CFT-positive cow, may be due to low bacterial levels in the early stage of infection (O'Leary et al., 2006) or infection that was cleared (Gwida et al., 2011). ITS-PCR was used in this study to detect *Brucella* spp. in cattle tissues and tissue homogenate cultures. The assay has been reported to detect extremely low levels of *Brucella* DNA in tissues (Keid et al., 2007), including tissues from seronegative animals (Kolo et al., 2019). In this study, the high sensitivity of ITS-PCR was demonstrated by the detection of *Brucella* DNA in mixed cultures, in which the concentration of bacteria was low. The ITS-PCR *Brucella* spp. detection rate of 2.9% determined by this study was lower than the detection rate of 12.5% reported at South African abattoirs (Kolo et al., 2019), which may reflect the differences in risk factors and prevalence of bovine brucellosis between the two countries. Results of this study show that ITS-PCR is a

useful tool for brucellosis surveillance, which needs to be validated for use on animal tissues by laboratories.

Considering that ITS-PCR can only identify *Brucella* to the genus level, the species-specific AMOS-PCR was used to confirm the isolation of *B. abortus* in Namibia from ten spleen and lymph node homogenate cultures. The findings by AMOS-PCR agreed with results of the culture and isolation method, confirming the widely reported sensitivity of molecular techniques (Karthik et al., 2014). To the best of our knowledge, this is the first isolation of *B. abortus* from cattle in Namibia. The isolation of *B. abortus* in cattle was not surprising since it is the most common cause of the disease in bovines worldwide. Due to the low bacterial concentration in tissues and subsequent low growth on culture plates, pure cultures could not be obtained for biotyping purposes. Since the AMOS-PCR only detects *B. abortus* biovar 1, 2 and 4 (Bricker and Halling, 1994), isolates in the current study can only be one of these biovars. In future, *B. abortus* S19-specific primers can be used to differentiate field and vaccine strains. As stated above, the *Brucella* strains isolated from male animals are most probably field strains, but this needs to be confirmed.

Despite the low seroprevalence and isolation rate of *Brucella* spp., it is of concern that a zoonotic pathogen was isolated from cattle tissues at a high throughput abattoir. Therefore, it is recommended that abattoir workers follow prescribed biosafety procedures including the wearing of adequate personal protective gear to prevent possible infection (Islam et al., 2013; Luwumba et al., 2019), because brucellosis does not present typical signs or lesions to permit the exclusion of affected cattle or meat at ante- or post-mortem inspection. It was encouraging that the study abattoir provided employees with adequate protective clothing for their specific responsibilities. Health education training is recommended in abattoir workers and meat handlers in general, to create awareness of and prevent brucellosis and other potential zoonoses.

There were limitations associated with the study. These include the fact that not all sera that were tested with RBT and CFT were subjected to the PCR assay, which may have underestimated the proportion of PCR-positive cases. Further, the low

concentration of brucellae in the tissues and cultures precluded the growth of pure cultures for biotyping. Therefore, it is imperative that future studies should confirm whether the strains of *B. abortus* isolated in Namibia are field or vaccine strains, using Bruce-ladder PCR (García-Yoldi et al., 2006) or S19- specific primers in an AMOS-PCR as described and recommended by Bricker and Halling (1995), since the vaccination of cattle against brucellosis is common in the country.

6.6. Conclusion

In this study, *Brucella abortus* was isolated from cattle tissues and a low seroprevalence of bovine brucellosis was determined at a major abattoir in Namibia. There is a risk of exposure to *Brucella* infection among abattoir workers, especially if biosafety procedures are neglected by the workers or not enforced by the authority.

6.7. References

ANIMAL HEALTH REGULATIONS (AHR). 2018. Government Notice 358 of 2018. Animal Health Act 1 of 2011. URL https://laws.parliament.na/cms_documents/animal-health-act-1-of-2011---regulations-2018-358-d37ff9f7a0.pdf.

AKINSEYE, V.O., ADESOKAN, H.K., OGUGUA, A.J., ADEDOYIN, F.J., OTU, P.I., KWAGHE, A.V., KOLAWOLE, N.O., OKORO, O.J., AGADA, C.A., TADE, A.O., FALEKE, O.O., OKEKE, A.L., AKANBI, I.M., IBITOYE, M.M., DIPEOLU, M.O., DALE, E.J., LORRAINE, P., TAYLOR, A.V., AWOSANYA, E.A., CADMUS, E.O., STACK, J.A. & CADMUS, S.I. 2016. Sero-epidemiological survey and risk factors associated with bovine brucellosis among slaughtered cattle in Nigeria. *Onderstepoort Journal of Veterinary Research,* 83(1), a1002. DOI: 10.4102/ojvr.v83i1.1002.

AL DAHOUK, S., TOMASO, H., NÖCKLER, K., NEUBAUER, H. & FRANGOULIDIS, D. 2003. Laboratory based diagnosis of brucellosis – a review of the literature. Part II: serological tests for brucellosis. *Clinical Laboratory*, 49, 577–589.

ALTON, G.G., JONES, L.M., ANGUS, R.D. & VERGER, J.M. 1988. Techniques for the Brucellosis Laboratory. Institut National de la Recherche Agronomique, Paris, France. pp. 81-134.

AWAH-NDUKUM, J., MOUICHE, M.M.M., KOUONMO-NGNOYUM, L., BAYANG, H.N., MANCHANG, T.K., POUEME, R.S.N., KOUAMO, J., NGUNGWA, V., ASSANA, E., FEUSSOM, K.J.M. & ZOLI, A.P. 2018. Seroprevalence and risk factors of brucellosis among slaughtered indigenous cattle, abattoir personnel and pregnant women in Ngaoundéré, Cameroon.*BMC Infectious Dis*ease, 18, 611. DOI: 10.1186/s12879-018-3522-x.

BANJO, T.A., OGUNDAHUNSI, O., OLOOTO, W.E., FAMILONI, O. & OYELEKAN, A.A.A. 2013. Occupational health hazards among abattoir workers in Abeokuta. *Academia Arena*, 5(10), 1-9.

BERCOVICH, Z. 1998. Maintenance of Brucella abortus free herds: a review with emphasis on epidemiology and the problems of diagnosing brucellosis in areas of low prevalence. *Veterinary Quarterly*, 20, 81-88. DOI: 10.1080/01652176.1998.9694845.

BERGER, S. 2016. Brucellosis: Global Status. Los Angeles, CA: GIDEON

Informatics, Inc.

BERHE, G., BELIHU, K. & ASFAW, Y. 2007. Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. *International Journal of Applied Research in Veterinary Medicine,* 5, 65-71.

BISHOP, G.C. 1984. A brucellosis serological survey on beef cattle at Cato Ridge Abattoir. *Journal of the South African Veterinary Association,* 55 (4), 185-186

BISHOP, G.C., BOSMAN, P.P. & HERR, S. 1994. Bovine brucellosis. In J.A.W. COETZER, G.R. THOMSON & R.C. TUSTIN (eds.), Infectious Diseases of livestock with special reference to Southern Africa, Oxford University Press, Cape Town. pp. 1053-1066.

BRICKER, B. J. & HALLING, S. M. 1994. Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis, Brucella ovis*, and *Brucella suis* bv. 1 by PCR. *Journal of Clinical Microbiology*, *32*(11), 2660–2666. DOI: 10.1128/jcm.32.11.2660-2666.1994.

BRICKER, B.J. & HALLING, S.M. 1995. Enhancement of the *Brucella* AMOS PCR assay for differentiation of *Brucella abortus* vaccine strains S19 and RB51. *Journal of Clinical Microbiology*, *33*(6), 1640–1642. DOI:10.1128/JCM.33.6.1640-1642.1995

CADMUS, S.I.B., ALABI, P.I., ADESOKAN, H.K., DALE, E.J. & STACK, J.A. 2013. Serological investigation of bovine brucellosis in three cattle production systems in Yewa Division, south-western Nigeria. *Journal of the South African Veterinary Association*, 84, 217. DOI: 10.4102/jsava.v84i1.217.

CADMUS, S.I.B., IJABONE, I.F., OPUTA, H.E., ADESOKAN, H.K. & STACK, J.A. 2006. Serological survey of brucellosis in livestock animal and workers in Ibadan Nigeria. *African Journal of Biomedical Research*, 9, 163-168.

CARVALHO NETA, A.V., MOL, J.P., XAVIER, M.N., PAIXÃO, T.A., LAGE, A.P. & SANTOS, R.L. 2010. Pathogenesis of bovine brucellosis. *Veterinary Journal*, 184, 146–155. DOI: 10.1016/j.tvjl.2009.04.010.

CHAKA, H., ABOSET, G., GAROMA, A., GUMI, B. & THYS, E. 2018. Cross-sectional survey of brucellosis and associated risk factors in the livestock-wildlife interface area of Nechisar National Park, Ethiopia. *Tropical Animal Health and Production*, *50*(5), 1041–1049. DOI: 10.1007/s11250-018-1528-4

CORBEL, M.J. 1997. Brucellosis. An overview. *Emerging Infectious Diseases*, 3(2), 213–221. DOI: 10.3201/eid0302.970219.

DÍAZ-APARICIO, E., MARÍN, C., ALONSO-URMENETA, B., ARAGÓN, V., PÉREZ-ORTIZ, S., PARDO, M. & MORIYÓN, I. 1994. Evaluation of serological tests for diagnosis of *Brucella melitensis* infection of goats. *Journal of Clinical Microbiology*, 32, 1159-1165. DOI: 10.1128/jcm.32.5.1159-1165.1994.

DVS (Directorate of Veterinary Services). 1987. Annual Report. South West Africa.

DEKA, R.P., MAGNUSSON, U., GRACE, D. & LINDAHL, J. 2018. Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India- a review. *Infection Ecology and Epidemiology*, 8, 1, DOI:10.1080/20008686.2018.1556548.

EC (European Commission). 2001. Brucellosis in sheep and goats *(Brucella melitensis).* SANCO.C.2/AH/R23/2001. European Union: Health and Consumer Protection Directorate General. URL https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out59_en.pdf

EL-DIASTY, M., WARETH, G., MELZER, F., MUSTAFA, S., SPRAGUE, L. D. & NEUBAUER, H. 2018. Isolation of *Brucella abortus* and *Brucella melitensis* from seronegative cows is a serious impediment in brucellosis control. *Veterinary Sciences*, *5*(1), 28. DOI: 10.3390/vetsci5010028.

GARCÍA-YOLDI, D., MARÍN, C.M., DE MIGUEL, M.J., MUÑOZ, P.M., VIZMANOS, J.L. & LÓPEZ-GOÑI. I. 2006. Multiplex PCR assay for the identification and differentiation of all *Brucella* species and the vaccine strains *Brucella abortus* S19 and RB51 and *Brucella melitensis* Rev 1. *Clinical Chemistry*, 52, 779-781. DOI: 10.1373/clinchem.2005.062596.

GARIN-BASTUJI, B., BLASCO, J.M., GRAYON, M. & VERGER, J.M. 1998. *Brucella melitensis* infection in sheep: present and future. *Veterinary Research,* 29, 255-274.

GODFROID, J. 2002. Brucellosis in wildlife. *Revue Scientifique et Technique-Office International des Epizooties,* 21(2), 277–286. DOI: 10.20506/rst.21.2.1333.

GODFROID, J., BISHOP, G.C., BOSMAN, P.P. &HERR, S. 2004. Bovine brucellosis. In: COETZER, J.A.W., TUSTIN, R.C., Eds., Infectious diseases of livestock. Cape Town, South Africa, Oxford University Press, p. 1510-1527.

GODFROID, J., CLOECKAERT, A., LIAUTARD, J.P., KOHLER, S., FRETIN, D., WALRAVENS, K., GARIN-BASTUJI, B. & LETESSON, J.J. 2005. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Veterinary Research*, 36(3), 313–326. DOI: 10.1051/vetres:2005003.

GODFROID, J., GARIN-BASTUJI, B., SAEGERMAN, C. & BLASCO, J.M. 2013a. Brucellosis in terrestrial wildlife. *Revue Scientifique et Technique (International Office of Epizootics),* 32, 27–42. DOI: 10.20506/rst.32.1.2180.

GODFROID, J., AL DAHOUK, S., PAPPAS, G., ROTH, F., MATOPE, G., MUMA, J., MARCOTTY, T., PFEIFFER, D., SKJERVE, E. 2013b. A "One Health" surveillance and control of brucellosis in developing countries: moving away from improvisation. *Comparative Immunology, Microbiology and Infectious Diseases*, 36(3), 241-8. DOI: 10.1016/j.cimid.2012.09.001.

GWIDA, M.M., EL-GOHARY, A.H., MELZER, F., TOMASO, H., RÖSLER, U., WERNERY, U., WERNERY, R., ELSCHNER, M.C., KHAN, I., EICKHOFF, M., SCHÖNER, D. & NEUBAUER, H. 2011. Comparison of diagnostic tests for the detection of *Brucella* spp. in camel sera. *BMC Research Notes*, *4*, 525. DOI: 10.1186/1756-0500-4-525.

HALLING, S.M., PETERSON-BURCH, B.D., BRICKER B.J., ZUERNER, R.L., QING, Z., LI, L-L., KAPUR, V., ALT, D.P. & OLSEN, S.C. 2005. Completion of the genome sequence of *Brucella abortus* and comparison to the highly similar genomes of *Brucella melitensis* and *Brucella suis*. *Journal of Bacteriology,* 187(8), 2715-2726. DOI:10.1128/JB.187.8.2715-2726.2005.

ISLAM, M.A., KHATUN, M.M., WERE, S.R., SRIRANGANATHAN, N. & BOYLE, S.M. 2013. A review of *Brucella* seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. *Veterinary Microbiology*, 166(3-4), 317-326. DOI: 10.1016/j.vetmic.2013.06.014.

KARTHIK, K., RATHORE, R., THOMAS, P., ELAMURUGAN, A., ARUN, T.R. & DHAMA, K. 2014. Serological and molecular detection of *Brucella abortus* from cattle by RBPT, STAT and PCR and sample suitability of whole blood for PCR. *Asian Journal of Animal and Veterinary Advances*, 9, 262-269. DOI: 10.3923/ajava.2014.262.269.

KEBEDE, T., EJETA, G. & AMENI, G. 2008. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida District). *Revue de'Elevage et Médecine Vétérinaire des Pays Tropicaux,* 159, 3–9.

KEID, L., SOARES, R., VIEIRA, N., MEGID, J., SALGADO, V., VASCONCELLOS, S., DA COSTA, M., GREGORI, F. & RICHTZENHAIN, L.J. 2007. Diagnosis of canine brucellosis: comparison between serological and microbiological tests and a PCR based on primers to 16S-23S rDNA interspacer. *Veterinary Research Communications*, 31(8), 951–965. DOI: 10.1007/s11259-006-0109-6.

KOLO, F.B., ADESIYUN, A.A., FASINA, F.O., KATSANDE, C.T., DOGONYARO, B.B., POTTS, A., MATLE, I., GELAW, A.K. & VAN HEERDEN, H. 2019. Seroprevalence and characterization of *Brucella* species in cattle slaughtered at Gauteng abattoirs, South Africa. *Veterinary Medicine and Science*, 00, 1–11. DOI: 10.1002/vms3.190.

JIMENEZ DE BAGUES, M.P., MARIN C. & BLASCO J.M. 1991. Effect of antibiotic therapy and strain 19 vaccination on the spread of *Brucella melitensis* within an infected dairy herd. *Preventive Veterinary Medicine*, 11, 17-24. DOI: 10.1016/S0167-5877(05)80041-8.

LEDWABA, M.B., GOMO, C., LEKOTA, K.E., LE FLÈCHE, P., HASSIM, A., VERGNAUD, G. & VAN HEERDEN, H. 2019. Molecular characterization of *Brucella* species from Zimbabwe. *PLoS Neglected Tropical Diseases*, 13(5), e0007311. DOI: 10.1371/journal.pntd.0007311.

LEDWABA, M.B., NDUMNEGO, O.C., MATLE, I., GELAW, A.K. & VAN HEERDEN, H. 2020. Investigating selective media for optimal isolation of *Brucella* spp. in South Africa', *Onderstepoort Journal of Veterinary Research* 87(1), a1792. DOI: 10.4102/ojvr.v87i1.1792.

LUWUMBA, D., KUSILUKA, L. & SHIRIMA, G. 2019. Occupational hazards associated with human brucellosis in abattoir settings: A case study of Dodoma abattoir in Tanzania. *Journal of Veterinary Medicine and Animal Health*, 11(3), 73-80. DOI: 10.5897/JVMAH2019.0752.

MADZINGIRA, O., FASINA, F.O., KANDIWA, E., MUSILIKA-SHILONGO, A., CHITATE, F. & VAN HEERDEN, H. 2020. A retrospective sero-epidemiological survey of bovine brucellosis on commercial and communal

farming systems in Namibia from 2004 to 2018. *Tropical Animal Health and Production*. DOI: 10.1007/s11250-020-02332-4.

MADZINGIRA, O. & MCCRINDLE, C. 2014. Prevalence of *Brucella* antibodies in sheep and springbok (*Antidorcas marsupialis*) reared together in the Karas region, Namibia. *Bulletin of Animal Health and Production in Africa*, 62, 299-306.

MADZINGIRA, O. & MCCRINDLE, C. 2015. Retrospective analysis of the prevalence of *Brucella* antibodies in sheep in the Karas Region of Namibia. *Tropical Animal Health and Production* 47, 1117. DOI:10.1007/s11250-015-0838-z.

MADZINGIRA, O. & MCCRINDLE, C. 2016. A questionnaire survey of risk factors of brucellosis on mixed sheep and springbok (*Antidorcas marsupialis*) farms. *International Science and Technical Journal of Namibia,* 8, 43-49.

MADZINGIRA, O. & SEZUNI, M.P. 2017. Serological prevalence and public health significance of brucellosis on a dairy farm in Namibia from 2011-2014*. BMC Research Notes* 10, 620. DOI: 10.1186/s13104-017-2933-x.

MAGWEDERE, K., BISHI, A., TJIPURA-ZAIRE, G., EBERLE, G., HEMBERGER, Y., HOFFMAN, L.C. AND & DZIVA, F. 2011. Brucellae through the food chain: the role of sheep, goats and springbok (*Antidorcus marsupialis*) as sources of human infection in Namibia. *Journal of the South African Veterinary Association*, 82, 205-212. DOI: 10.4102/jsava.v82i4.75.

MANGEN, M-J, OTTE, J., PFEIFFER, D. & CHILONDA, P. 2002. Bovine brucellosis in Sub-Saharan Africa: estimation of sero-prevalence and impact on meat and milk offtake potential. Food and Agricultural Organisation, Rome, Italy.

MARTIN, S.W., MEEK, A.H. & WILLENBERG, P. 1987. *Veterinary epidemiology: principles and methods (1st edn).* Iowa: Iowa State University Press.

MATOPE, G., BHEBHE, E., MUMA, J.B., OLOYA, J., MADEKUROZWA, R.L., LUND, A. & SKJERVE, E. 2011. Seroprevalence of brucellosis and its risk in cattle from smallholder dairy farms in Zimbabwe. *Tropical Animal Health and Production,* 43, 975–979. DOI: 10.1007/s11250-011-9794-4.

MCDERMOTT, J.D., GRACE, D. & ZINSSTAG, J. 2013. Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique-Office International des Epizooties*, 32(1), 249–61.

DOI: 10.20506/rst.32.1.2197.

MCDERMOTT, J.J. & ARIMI, S.M. 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary Microbiology*, 90(1–4), 111–134. DOI: 10.1016/s0378-1135(02)00249-3.

MEAT BOARD. 2019. Integrated annual report 2018/19. Meat Board of Namibia. URL http://www.nammic.com.na

MERGESA, B., BIFFA, D., NIGUSE, F., RUFAE, T., ASMARE, K. & SKJERVE, E. 2011. Cattle brucellosis in traditional livestock husbandry practice in Southern and Eastern Ethiopia, and its zoonotic implication. *Acta Veterinaria Scandinavia*, 53, 24-31. DOI: 10.1186/1751-0147-53-24.

MIONI, M. DES .R., VICENTE, A.F., PERES, M.G., APPOLINARIO, C.M., RIBEIRO B.L.D., PANTOJA, J.C.DE F., PINTO, J.P.DEA.N., MATHIAS, L.A. & MEGID, J. 2018. Brucellosis prevalence in Brazilian slaughterhouses with different meat inspection systems. *Journal of Food Protection*, 81, 1073-1078. DOI: 10.4315/0362-028XJFP-17-451.

MIRAMBO, M.M., MGODE, G.F., MALIMA, Z.O., JOHN, M., MNGUMI, E.B., MHAMPHI, G.G. & MSHANA, S.E. 2018. Seropositivity of *Brucella* spp. and *Leptospira* spp. antibodies among abattoir workers and meat vendors in the city of Mwanza, Tanzania: A call for one health approach control strategies. *PLoS Neglected Tropical Diseases*, 12(6), e0006600. DOI: 10.1371/journal.pntd.0006600.

MOHAN, K., MAKAYA, P.V., MUVAVARIRWA, P., MATOPE, G., MAHEMBE, E. & PAWANDIWA, A. 1996. Brucellosis surveillance and control in Zimbabwe: bacteriological and serological investigation in dairy herds. *Onderstepoort Journal of Veterinary Research*, 63, 47–51.

MORENO, E. 2002. Brucellosis in Central America. *Veterinary Microbiology,* 90, 31-38.

MUKHTAR, F. 2010. Brucellosis in a high risk occupational group: Seroprevalence and analysis of risk factors. *Journal of the Pakistan Medical Association*, 60(12), 1031-1034.

MUMA, J.B., SAMUI, K.L., SIAMUDAALA, V.M., OLOYA, J., MATOPE, G., OMER, M.K., MUNYEME, M., MUBITA, C. & SKJERVE, E. 2006. Prevalence of antibodies to *Brucella* spp. and individual risk factors of infection in traditional

cattle, goats and sheep reared in livestock–wildlife interface areas of Zambia. *Tropical Animal Health and Production*, 38, 195-206. DOI: 10.1007/s11250-006-4320-9.

OIE. 2018. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 3.1.4. Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis) (infection with B. abortus, B. melitensis* and *B. suis*). URL http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf.

O'LEARY, S. & SHEAHAN, M. & SWEENEY, T. 2006. *Brucella abortus* detection by PCR assay in blood, milk and lymph tissue of serologically positive cows. *Research in Veterinary Science*, 81, 170–6. DOI: 10.1016/j.rvsc.2005.12.001.

PAPPAS, G. 2010. The changing *Brucella* ecology: novel reservoirs, new threats. *International Journal of Antimicrobial Agents*, 36, S8-S11. DOI: 10.1016/j.ijantimicag.2010.06.013.

SELIM, A., ATTIA, K., RAMADAN, E., HAFEZ, Y. M. & SALMAN, A. 2019. Seroprevalence and molecular characterization of *Brucella* species in naturally infected cattle and sheep. *Preventive Veterinary Medicine*, *171*, 104756. DOI: 10.1016/j.prevetmed.2019.104756.

SHAFEE, M., RABBANI, M., SHEIKH, A.A., AHMAD, M.D. & RAZZAQ, A. 2011. Prevalence of bovine brucellosis in organized dairy farms, using milk ELISA, in Quetta City, Balochistan, Pakistan. *Veterinary Medicine International*. DOI: 10.4061/2011/358950.

SHEY-NJILA, O., DAOUDA, E., NYA, E., ZOLI, P.A., WALRAVENS, K., GODFROID, J. & GEERTS, S. 2005. Serological survey of bovine brucellosis in Cameroon. *Revue d'Elevage et de Médicine Véterinaire des Pays Tropicaux*, 58, 139-143.

SPICKLER, A.R. 2018. Brucellosis: *Brucella abortus*. URL http://www.cfsph.iastate.edu/DiseaseInfo/ factsheets.php.

SWAI, E.S. & SCHOONMAN, L. 2009. Human brucellosis: seroprevalence and risk factors related to high risk occupational groups in Tanga Municipality, Tanzania. *Zoonoses and Public Health*, 56, 183–187. DOI: 10.1111/j.1863-2378.2008.01175.x.

TALESKI, V. 2010. An overview of introducing various laboratory tests for diagnosis

of human brucellosis in the Republic of Macedonia. *Macedonian Journal of Medical Sciences*, 3, 239-245. DOI: 10.3889/MJMS.1857-5773.2010.0135.

TOLOSA, T., REGASSA, F. & BELIHU, K. 2008. Seroprevalence study of bovine brucellosis in extensive management system in selected sites of Jimma Zone, Western Ethiopia. *Bulletin of Animal Health and Production in Africa,* 56, 25–37.

VERGER, J.M., GRIMONT, F., GRIMONT, P.A.D. & GRAYON, M. 1985. *Brucella*, a monospecific genus as shown by deoxyribonucleic acid hybridization. International *Journal of Systematic and Evolutionary Microbiology*, 35 (3). DOI: 10.1099/00207713-35-3-292.

WEINER, M., IWANIAK, W., & SZULOWSKI, K. 2011. Comparison of PCR based AMOS, Bruce-Ladder and MLVA assays for typing of *Brucella* species. *Bulletin of the Veterinary Institute in Pulawy*, *55*, 625–630.

WHO. 1997. Fact sheet N173. Geneva, Switzerland: World Health Organization.

WHO. 2006. Brucellosis in humans and animals. Food and Agriculture Organization of the United Nations, World Health Organization and World Organisation for Animal Health. Brucellosis in humans and animals. World Health Organization. URL https://apps.who.int/iris/handle/10665/43597.

WHO. 2011. Seven neglected endemic zoonoses-some basic facts. Geneva: World Health Organization. URL http://www.who.int/zoonoses/neglectedzoonoticdiseases/en/.

Chapter 7: General Discussion, Recommendations and Conclusions

7.1. General discussion

Results of this study showed that the seroprevalence of bovine brucellosis in the cattle farming system (Chapter 5) and at the abattoir (Chapter 6) were low (0.49% and 1.64% respectively) and similar to prevalence (0.0%, 0.01% and 0.50%) reported by previous studies in Namibia (DVS, 1987; Magwedere et al., 2011; Madzingira and Sezuni, 2017). Likewise, the proportion of infected herds in the retrospective study (9.26%) and at the abattoir (9.60%) were similar and low. Brucellosis seroprevalence was higher at the abattoir (prospective study) than in the cattle farming system (retrospective study), most probably due to a smaller sample size (n = 304) compared to the retrospective study (n = 49 718). Most cattle in the current study originated from commercial farming areas where vaccination against brucellosis using *B. abortus* S19 vaccine is frequent, which could have overestimated the prevalence of anti-*Brucella* antibodies. Individual cattle and herd prevalence of brucellosis in the retrospective study, were higher in the communal areas (10.27% and 33.09%) than in the commercial farming areas (0.24% and 4.67%), even though most of the cattle tested were from commercial (n = 48 462) than communal (1256) areas. These findings show that brucellosis is a bigger challenge in communal than in commercial cattle herds, probably due to the high frequency of risk factors for the disease in the communal areas such as low vaccination rates (Chapter 3) and the mingling of different cattle herds (Madzingira et al., 2020). Further, the study on knowledge, attitudes, and practices regarding brucellosis (Chapter 3) revealed that communal livestock farmers had significantly lower levels of brucellosis knowledge than commercial livestock farmers and as a result, they were unlikely to control brucellosis in their cattle herds or protect themselves against infection. Despite the relatively high bovine brucellosis prevalence recorded in the communal areas, a clear decline in the number of positive reactors per year was observed. Whether this is due to improvements in the accessibility of veterinary services in the communal areas in recent years or low levels of cattle testing needs further investigation. The low animal prevalence determined in commercial dairy cattle (0.19%) and beef cattle (0.30%) is an

endorsement of the effectiveness of brucellosis control measures implemented on commercial cattle farms. According to Madzingira et al. (2020), control of brucellosis on commercial cattle farms is strictly enforced by veterinary officials. Furthermore, commercial dairy cattle are tested annually and any reactors are culled as per the Animal Health regulations (2018).

In the retrospective seroprevalence study in cattle (Chapter 5), all cows that aborted on commercial farms were seronegative, perhaps due to undetectable antibody levels that are associated with the period immediately following abortions (Mittal et al., 2018). However, this is unlikely to have been the case, because 12.65% of abortion-linked sera in the communal areas were seropositive. Further, in all cases of abortions, diagnosis at the laboratory was routinely complimented by examination of modified Ziehl-Nielsen stained smears of abortion material and conventional PCR for the identification of *Brucella* DNA. These findings suggest that brucellosis is not a frequent cause of abortions in commercial cattle herds in Namibia. Therefore, other potential causes of abortions in cattle, such as *Chlamydophila abortus, Coxiella burnetii*, *Campylobacter fetus venerealis* and *Leptospira* spp. need to be considered routinely in any diagnostic workup for abortion cases.

Bovine brucellosis cases were distributed in all administrative regions of Namibia, except Erongo and Kunene regions, while human brucellosis incident cases were recorded in all 14 regions of the country. Erongo and Kunene regions have predominantly hot and dry desert conditions and sparse cattle populations, which is not ideal for the survival and spread of *Brucella* infection between cattle or cattle herds. Whereas bovine brucellosis cases were clustered in the northern and north-eastern communal areas of the country, there were more human cases in the commercial cattle and sheep farming regions (Khomas, Hardap, Kunene and Karas), where seroprevalence of brucellosis in cattle has been determined to be low, the human population sparse and awareness of the disease is high (Chapter 3). The clustering of human brucellosis cases in the commercial livestock farming areas may be related to the large numbers of sheep and goats that are reared in these regions than in the communal areas. It is also suspected that the referral of complicated chronic brucellosis cases to the major hospitals in the commercial cattle farming

regions of the country may have played a role. Further studies are required to explain this finding.

In agreement with the endemic nature of the disease in cattle, the retrospective brucellosis study in humans (Chapter 4) determined seroprevalence of human brucellosis (11.64%) among patients presented at medical facilities around the country from 2012 to 2017. The prevalence of human cases doubled over the study period, despite a decline in presumptive cases and a relatively stable prevalence of bovine brucellosis during the same period. This may be due to low brucellosis awareness level (43.5%) (Chapter 3) and subsequent involvement in risky practices for *Brucella* infection by exposed professions such as farmers and meat handlers, as noted by the study on brucellosis knowledge, attitudes and awareness (Chapter 3). In the retrospective study in humans, suspected clinical cases of brucellosis were subjected to serological testing, which may have overestimated the observed prevalence. However, the higher prevalence estimated in humans (11.64%) than cattle (0.49%) is not surprising, because raw milk, the main source of infection for humans can be consumed by many people at once, resulting in an exponential rise in cases (Leong et al., 2015). The rapid diagnostic test that is currently used to screen for human brucellosis in Namibia has a low sensitivity (36.6%) and specificity (69.3%) (Kiambi et al., 2020). As a result, the use of this test in combination with tests of a higher sensitivity such as RBT, SAT or Coomb's test is recommended to prevent false negative cases that would not be placed on treatment. Since most human brucellosis cases (56.3%) were reported at private medical facilities, public medical facilities that serve an even larger number of patients need to upscale their surveillance for the disease. In Namibia, livestock herding, handling, and milking are primary responsibilities of males and this would suggest a higher level of exposure to brucellosis among males. However, results of the study showed more seropositive females (64.00%) than males, perhaps due to handling contaminated animal products such as meat at home or at abattoirs. Human brucellosis prevalence increased with age but was higher in the economically active age group (30-40 years), as was also observed in Namibia by Magwedere et al. (2011). In general, the young have limited contact with livestock, compared to adults, hence the observed trends. The data that were used in this study lacked information on clinical symptoms

at presentation and exposure factors. Therefore, risk factors for the disease could not be explored extensively.

This is the first report of the molecular characterisation of *B. abortus* from mixed (non-pure) cultures of cattle tissues (spleen and lymph nodes) in Namibia based on AMOS-PCR (Chapter 6). Previous studies, using serological tests, could only identify the bacteria to genus level. The isolation of *B. abortus* from cattle was expected because cattle are the primary reservoir host for this species (Verger et al., 1982). The study used RBT and CFT in series to identify seropositive cattle; ITS-PCR to confirm the presence of *Brucella* DNA from tissue homogenate cultures and AMOS-PCR to identify *Brucella* isolates. The *Brucella* DNA detection rate using ITS-PCR among the tested tissues was low (2.9%) and similar to the low prevalence reported in the retrospective bovine seroprevalence study (0.49%) and the seroprevalence study at the abattoir (1.64%). *Brucella* isolates were from spleens (85.7%, 6/7) and lymph nodes (57.1%, 4/7), confirming these tissues as valuable samples for bacterial culture and isolation of *Brucella* (OIE, 2018). However, the isolates could not be typed due to a failure to obtain pure cultures because of the low concentration of bacteria in the tissues. Future studies should attempt purification and biotyping of the species as well as whole genome sequencing to characterize the isolates, which will improve the understanding of transmission patterns and risk factors for the disease (Mathew et al., 2015).

Knowledge, attitude, and awareness deficiencies that are a risk for *Brucella* infection were identified among farmers, meat handlers and medical professionals (Chapter 3), which may partly explain the relatively high prevalence of human brucellosis (11.64%) determined in this study (Chapter 4). Although commercial farmers and medical professionals were highly knowledgeable about brucellosis, they engaged in practices that pose a risk for *Brucella* infection, such as drinking raw milk, handling animals, or assisting delivery with bare hands. Therefore, impartation of knowledge alone may not be sufficient to protect individuals from infection. Rather, interventions targeted at changing attitudes and behaviours should be included to ensure that individuals do not engage in practices that are a risk for *Brucella* infection. Knowledge of brucellosis was low among communal farmers and meat handlers and

therefore, these groups should be targeted during public health education campaigns. Brucellosis was rarely considered as part of the differential diagnoses for cases of persistent fever in humans despite a high frequency of medical professionals that had knowledge of the disease. As a result, brucellosis may be under-diagnosed at medical facilities around the country, placing patients at risk of the severe and debilitating chronic form of the infection. Some respondents indicated that abortion materials were left on pastures or fed to dogs. This has the potential to contaminate the environment with *Brucella* bacteria, especially as dogs have been reported to play an important role in the epidemiology of bovine brucellosis by dragging aborted material around the farm (Forbes, 1990).

7.2. Recommendations

Results of this study confirm that the prevalence of bovine brucellosis in Namibia is low. Despite the low proportion of infected cattle and cattle herds reported in this study, the zoonotic nature of the disease and the relatively high prevalence determined in humans necessitate the implementation of appropriate prevention and control measures. According to Nicoletti (1993), in regions where bovine brucellosis seroprevalence is less than 2% as in this study, an eradication strategy based on the test and slaughter approach is recommended. However, the financial implications of this strategy are high, especially the cost of testing cattle and compensating farmers for culled seropositive animals. Since the current bovine brucellosis control measures are less costly, but effective in keeping seroprevalence at a low level (Madzingira et al., 2020), it is recommended that they be maintained, but strictly enforced especially in the communal areas where relatively higher seroprevalence was determined. Widespread application of vaccination of cattle in the communal areas is recommended to reduce the incidence and transmission of the disease. It is also recommended that the current voluntary vaccination of sheep against brucellosis be promoted among sheep farmers and the consumption of raw sheep and goat milk be discouraged, as these are potential sources of human infection (Musallam et al., 2015). The capacity for veterinary and public health officials to carry out disease surveillance, engage and provide extension services to communities should be enhanced for better brucellosis prevention and management of the disease (Dadar et al., 2021). Bovine brucellosis control measures in Namibia are

outlined in the Animal Health Regulations (2018) and include compulsory once-off vaccination of heifers from 3 to 8 months with S19 vaccines or female cattle above 8 months of age with RB51 vaccines, serological testing of milk-producing cattle at least once a year, importation of brucellosis-free cattle, identification of seropositive cattle and herds, quarantine measures, and slaughter of positive cattle under veterinary supervision.

Despite the low seroprevalence and pathogen isolation rate in cattle, it is of concern that *B. abortus*, a zoonotic pathogen was isolated at a high throughput abattoir. Therefore, abattoir and butchery owners should provide regular training to meat and animal handlers on zoonotic diseases and their prevention as part of their occupational health and safety training programs and appropriate protective gear to protect against *Brucella* infection. Although brucellae have a short survival time in meat, the handling of meat with compromised skins in the slaughterhouse, butchery or kitchen should be avoided to prevent wound inoculation of bacteria. For the meat consumer, thorough cooking of meat and the boiling of milk before consumption are the best options for preventing *Brucella* infection. Since, *Brucella* tend to concentrate in lymph nodes, careful removal of lymph nodes may reduce the chances of infection, but survival of the bacteria in meat is limited due to the decline in pH post-mortem (EC, 2001). In humans, brucellosis can also be brought under control based on measures implemented in the livestock sector (Vemulapali et al., 2004). However, these measures alone may not be adequate, as inappropriate practices and attitudes as observed in this study (Chapter 3) can expose individuals to infection. Considering this along with the relatively high prevalence of human brucellosis recorded in this study, a One Health approach with collaboration between public health and veterinary officials (Dadar et al., 2021) is proposed. The strategy, should use health education and risk communication strategies as suggested by the respondents to disseminate knowledge on disease risk factors and prevention measures to the public in general and persons in the animal and meat industries specifically. In particularly, the pasteurization or boiling of raw milk before consumption (Kiambi et al., 2020) or using it in yoghurt production and the adoption of safe meat and animal handling practices should be promoted (Islam et al., 2013; Luwumba et al., 2019). According to the FAO (2010), human populations that are

aware of the epidemiological aspects of brucellosis and prevention methods, have a reduced risk of *Brucella* infection. Health education targeted at occupationally exposed groups such as farmers, butchers and abattoir workers, and resource poor communities is a potent tool for raising awareness of the socioeconomic effects of the disease and preventive measures against *Brucella* infection (Marcotty et al., 2009; FAO, 2010). It is recommended that regular continuous professional training be provided to medical professionals on zoonotic diseases such as brucellosis to improve surveillance for the disease at medical facilities in the country. Future studies to determine the burden of or exposure to *Brucella* infection in occupationally exposed groups are recommended.

7.3. Conclusions

Results from this project showed that the communal farmers and meat handlers had low levels of brucellosis knowledge. Knowledge, attitude, and practice gaps that can promote *Brucella* infection in humans and cattle were identified among cattle farmers, meat handlers and medical professionals. However, positive attitudes, such as the desire by participants to receive more information on brucellosis, are opportunities that can be utilised to disseminate health education. Brucellosis seroprevalence in cattle in the country, at the abattoir and in humans was 0.49%, 1.64% and 11.64% respectively. Human brucellosis cases were reported in all 14 regions of the country, but were more in the commercial cattle and sheep farming regions of Khomas, Hardap, Kunene and Karas than other regions of the country. Bovine brucellosis cases were clustered in the northern and north-eastern communal areas of the country. In this study, *B. abortus* was isolated and characterised from cattle lymph nodes and spleens using ITS-PCR and AMOS-PCR.

7.4. References

ANIMAL HEALTH REGULATIONS. 2018. Animal Health Regulations of the Animal Health Act 1 of 2011 (Government Notice 358 of 2018). https://laws.parliament.na/cms_documents/animal-health-act-1-of-2011---regulations-2018-358-d37ff9f7a0.pdf. *Accessed May 25, 2019*.

DADAR, M., TIWARI, R., SHARUN, K. & DHAMA, K. 2021. Importance of brucellosis control programs of livestock on the improvement of one health. *Veterinary Quarterly*, 41, 137-151. DOI: 10.1080/01652176.2021.1894501

EC (European Commission). 2001. Brucellosis in sheep and goats *(Brucella melitensis).* SANCO.C.2/AH/R23/2001. European Union: Health and Consumer Protection Directorate General. URL https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out59_en.pdf

DVS. 1987. Directorate of Veterinary Services Annual Report. South West Africa.

FAO. 2010. *Brucella melitensis* in Eurasia and the Middle East. FAO Animal Production and Health Proceedings No. 10. Rome, Italy. http://www.fao.org/3/i1402e/I1402E.pdf.

FORBES, L.B. 1990. *Brucella abortus* infection in 14 farm dogs. *Journal of the American Veterinary Medical Association*, 196: 911-6.

ISLAM, M.A., KHATUN, M.M., WERE, S.R., SRIRANGANATHAN, N. & BOYLE, S.M. 2013. A review of *Brucella* seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. *Veterinary Microbiology*, 166(3-4), 317-326. DOI: 10.1016/j.vetmic.2013.06.014.

KIAMBI, S.G., FÈVRE, E.M., OMOLO, J., OUNDO, J. & DE GLANVILLE, W.A. 2020. Risk factors for acute human brucellosis in Ijara, north-eastern Kenya. *PLoS Neglected Tropical Diseases*, 14(4), e0008108. DOI: 10.1371/journal.pntd.0008108.

LEONG, K.N., CHOW, T.S., WONG, P.S., HAMZAH, S.H., AHMAD, N., & CH'NG, C. C. 2015. Outbreak of human brucellosis from consumption of raw goats' milk in Penang, Malaysia. *The American Journal of Tropical Medicine and Hygiene*, *93*(3), 539–541. DOI:10.4269/ajtmh.15-0246

LUWUMBA, D., KUSILUKA, L. & SHIRIMA, G. 2019. Occupational hazards

associated with human brucellosis in abattoir settings: A case study of Dodoma abattoir in Tanzania. *Journal of Veterinary Medicine and Animal Health*, 11(3), 73-80.

MADZINGIRA, O. & SEZUNI, M.P. 2017. Serological prevalence and public health significance of brucellosis on a dairy farm in Namibia from 2011-2014*. BMC Research Notes* 10, 620. DOI: 10.1186/s13104-017-2933-x.

MADZINGIRA, O., FASINA, F.O., KANDIWA, E., MUSILIKA-SHILONGO, A., CHITATE, F. & VAN HEERDEN, H. 2020. A retrospective sero-epidemiological survey of bovine brucellosis on commercial and communal farming systems in Namibia from 2004 to 2018. *Tropical Animal Health and Production, 52*(6), 3099-3107. DOI: 10.1007/s11250-020-02332-4.

MARCOTTY, T., MATTHYS, F., GODFROID, J., RIGOUTS, L., AMENI, G., GEY VAN PITTIUS, N., KAZWALA, R., MUMA, J., VAN HELDEN, P., WALRAVENS, K., DE KLERK, L.M., GEOGHEGAN, C., MBOTHA, D., OTTE, M., AMENU, K., ABU SAMRA, N., BOTHA, C., EKRON, M., JENKINS, A., JORI, F., KRIEK, N., MCCRINDLE, C., MICHEL, A., MORAR, D., ROGER, F., THYS, E. & VAN DEN BOSSCHE, P. 2009. Zoonotic tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health issues? The outcomes of a multi-disciplinary workshop. *Annals of Tropical Medicine and Parasitology*, 103, 401-411. DOI: 10.1179/136485909X451771.

MATHEW, C., STOKSTAD, M., JOHANSEN, T.B., KLEVAR, S., MDEGELA, R.H., MWAMENGELE, G., MICHEL, P., ESCHOBAR, L., FRETIN, D. & GODFROID, J. 2015. First isolation, identification, phenotypic and genotypic characterization of *Brucella abortus* biovar 3 from dairy cattle in Tanzania. *BMC Veterinary Research,* 11, 156. DOI: 10.1186/s12917-015-0476-8.

MITTAL, M., SHARMA, V., NEHRA, K., CHAKRAVARTI, S., KUNDU, K., BANSAL, V.K., CHURAMANI, C.P. & KUMAR, A. 2018. Abortions in an organized dairy farm from North India reveal the possibility of breed susceptibility to bovine brucellosis. *One Health*, 5, 1-5. DOI:10.1016/j.onehlt.2017.11.001

MUSALLAM, I.I., SHEHADA, M.N.A. & GUITIAN, J. 2015. Knowledge, attitudes and practices associated with brucellosis in livestock owners in Jordan. *American Journal of Tropical Medicine and Hygiene*, 93(6), 1148-1155. DOI:10.4269/ajtmh.15-0294

NICOLETTI, P. 1993. The eradication of brucellosis in animals. *Saudi Medical Journal*, 14, 288-292.

OIE. 2018. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 3.1.4. Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis) (infection with B. abortus, B. melitensis* and *B. suis*). URL http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf.

VEMULAPALLI, R., CONTRERAS, A., SANAKKAYALA, N., SRIRANGANATHAN, N., BOYLE, S.M. & SCHURIG, G.G. 2004. Enhanced efficacy of recombinant *Brucella abortus* RB51 vaccines against *B. melitensis* infection in mice. *Veterinary Microbiology*, 102(3-4), 237-45. DOI: 10.1016/j.vetmic.2004.07.001.

VERGER, J.M., DUEE, J. & GRAYON, M. 1982. Brucella typing a ten-year investigation in France. *Annals of Microbiology (Inst. Pasteur)*, 133 B: 433- 47.

Appendices

Appendix 1: Brucellosis survey questionnaire: cattle farmers

Please give a response by placing an 'X' in the boxes or by filling in the blank spaces.

Date: _____ **Questionnaire number:** _____

SECTION A:

1. Age (tick as appropriate): 18-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ >60 ☐

2. Gender (tick as appropriate): Male ☐ Female ☐

3. Town and Region: _____

4. Highest education level:

- No formal education ☐
- Primary school level ☐
- Secondary school level ☐
- Certificate ☐
- Diploma ☐
- University degree ☐

5. Profession/farming system:

Cattle farmer ☐
Cattle farm manager ☐
Communal farmer ☐
Commercial farmer ☐

6. Number of years in the profession: _____

SECTION B:

7. List any diseases that can cause abortions in cattle (in any Namibian language):

_____

8. Have you heard of a disease called brucellosis? Yes ☐ No ☐

If yes in point 8 above, which animals can get brucellosis?

_____

9. Where did you hear about brucellosis? _____

10. Can people get brucellosis? Yes ☐ No ☐

If yes in point 10 above, how do humans get this disease? _____

11. How is brucellosis spread between cattle?

12. What are the symptoms of brucellosis in humans?

13. What signs do cattle affected by brucellosis show?

14. Is brucellosis a treatable disease in humans?Yes ☐ No ☐ I don't know ☐

15. How can brucellosis be prevented in:

15.1 People? _____

15.2 Cattle? _____

16. Do you know of any person that has had brucellosis? Yes ☐ No ☐

16.1 If yes in point 16 above, how were they infected? _____

16.2 If yes in point 16 above, were they treated at the hospital? _____

16.3 If yes in point 16 above, did the person die ☐ recover ☐ or is still sick ☐

17. Which of the following symptoms have you experienced in the past year?

Continuous fever ☐

Fever that comes and goes ☐

Profuse sweating at night ☐

Weakness ☐

Joint pains ☐

Muscle pains ☐

Headache ☐

Back pain ☐

18. Do you think you are at risk of infection with brucellosis? Yes ☐ No ☐

SECTION C:

19. Livestock species, breeds and numbers kept:

Livestock species	**Total number**	**Breed (s)**	**Breeding females**	**Breeding males**	**Calves, lambs or kids**
Cattle					
Pigs					
Sheep					
Goats					

20. How are cattle reared on your farm?

Cattle share grazing with sheep ☐

Cattle share grazing with goats ☐

Cattle share grazing with both sheep and goats ☐

Cattle are reared separately from sheep and goats ☐

Only cattle are reared ☐

21. Cattle share drinking water with: sheep? Yes ☐ No ☐
goats? Yes ☐ No ☐

22. Source of drinking water for animals:
Dam ☐
Borehole/underground ☐
River ☐
Other _____

23. Source of feed for cattle:
From outside the farm ☐
From inside the farm ☐

24. From which places do you buy cattle for your farm?
Other cattle farms Yes ☐ No ☐
From auctions Yes ☐ No ☐
Other countries Yes ☐ No ☐
No cattle are brought from outside ☐ No ☐
Other places (please specify): _____

25. Do you check the health of animals before you buy them?
No, i don't carry out any checks ☐
Yes, I ask the veterinarian to check and carry out tests ☐
Yes, i check the animals myself ☐

26. How often do your cattle mix with cattle from neighboring farms?
They always mix ☐
They don't mix at all, farm is fenced ☐
Sometimes ☐

27. Where do you sell cattle from your farm?

At auctions ☐
At the local abattoir ☐
At the export abattoir ☐
Export to other countries ☐
Other: _____

28. Do you sell cows' milk:

on the farm? Yes ☐ No ☐
outside the farm? Yes ☐ No ☐

29. Which animal's milk is consumed on the farm? Sheep ☐ Goat ☐ Cow ☐ ☐
None

30. The following products are made from fresh milk on the farm:

Cheese ☐ yoghurt ☐ none of these ☐ other products (specify): _____

31. Who assists cows to give birth? _____

32. Please complete the table below:

	2016	**2017**	**2018**
Number of cows that aborted			
Number of calves born dead			
Number of cows with retained placentas			
Number of cows that did not get pregnant			
Number, age and sex of cattle with swollen joints			
Number of cattle bought from outside the farm			

33. Which vaccines do you give to cattle?

_____

_____

34. Do you vaccinate cattle against brucellosis? Yes ☐ No ☐

If yes, which age and sex do you vaccinate? _____

If yes, which vaccine do you use against brucellosis in cattle? _____

35. Have your cattle tested positive for brucellosis in the past year? (Yes/No) _____

35.1 If yes, who tested the cattle? _____

35.2 If yes, what happened to the animals that tested positive to brucellosis?

_____

SECTION D:

36. Fresh cow's milk and home-made cheese are as healthy as packaged fresh milk and cheese from the supermarket: Yes ☐ No ☐

37. Is it necessary to boil raw milk from a cow before drinking it? Yes ☐ No ☐

38. How serious do you consider abortions in cows?

Very serious ☐ Serious ☐ Not important ☐

39. What do you do with cattle that get sick?

Treat myself ☐

Look for a veterinarian to assist ☐

Slaughter for meat ☐

Other: _____

40. Would you need more information about brucellosis? Yes ☐ No ☐

40.1 If yes in point 40 above, how would you like to receive information about brucellosis?

_____

SECTION E:

41. How do you milk cows?

Hand milking ☐ Machine milking ☐ Cows are not milked ☐

42. If cows are milked, who does the milking? _____

43. Are hands washed after milking cows? Yes ☐ No ☐

44. Do you drink raw cow's milk? Yes ☐ No ☐

45. Do you use raw cow's milk in tea? Yes ☐ No ☐

46. Do you prepare and eat home-made cheese? Yes ☐ No ☐

47. Do you handle aborted fetuses and placentas with your hands? Yes ☐ No ☐

47.1 If the answer to 47 above is no, how do you handle them? _____

48. What do you do with aborted fetuses and placentas? (tick as appropriate)

Leave on the pastures (do nothing) ☐

Burn ☐

Feed to dogs ☐

Bury ☐

Call veterinarian ☐

49. What would you do if you suspected an animal had brucellosis?

_____

50. How do you prepare your meat for consumption?

Eat raw ☐

Boil ☐

☐

Partly cook

Roast ☐

51. Which of the following do you eat?

Cattle testicles	Yes	☐	No	☐
Female reproductive parts	Yes	☐	No	☐
Raw milk	Yes	☐	No	☐
Uncooked liver	Yes	☐	No	☐
Uncooked blood	Yes	☐	No	☐
Uncooked fore stomachs	Yes	☐	No	☐

52. Breeding methods used:

Artificial insemination ☐

Own bulls ☐

Communal bulls ☐

Both artificial insemination and own bulls ☐

Appendix 2: Brucellosis survey questionnaire: abattoir workers/butchers

Please give a response by placing an 'X' in the boxes or by filling in the blank spaces.

Date: _____ **Questionnaire number:** _____

SECTION A:

1. Age (tick as appropriate): 18-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ >60 ☐

2. Gender (tick as appropriate): Male ☐ Female ☐

3. Abattoir name: _____

4. Highest education level (tick appropriate answer):

No formal education	☐	Primary school level	☐
Secondary school level	☐	Certificate	☐
Diploma	☐	University degree	☐

5. Profession/type of work:

Butcher	☐	Abattoir worker	☐

6. Job title and Department: _____

7. Brief description of job activities: _____

8. List of protective gear worn: _____

9. Number of years in the job: _____

SECTION B:

10. Have you heard of a disease called brucellosis? Yes ☐ No ☐

If yes in point 10 above, what is the cause of the disease? _____

If yes in point 10 above, which animals can get brucellosis? _____

11. Where did you hear about brucellosis? _____

12. Can brucellosis infect people? Yes ☐ No ☐

If yes in 12 above, how do humans get the disease? _____

13. What are the symptoms of brucellosis in humans?

_____

14. How is brucellosis spread in cattle? _____

15. What are the clinical signs in cattle infected with brucellosis?

_____

16. Is brucellosis a treatable disease in: people? Yes ☐ No ☐ I don't know ☐

cattle? Yes ☐ No ☐ I don't know ☐

17. How can brucellosis be prevented in:

17.1 People? _____

17.2 Cattle? _____

18. Have you seen a person suffering from brucellosis? Yes ☐ No ☐

19. Which of the following symptoms have you experienced in the past year?

- Fever that comes and goes ☐
- Profuse sweating at night ☐
- Weakness ☐
- Joint pains ☐
- Muscle pains ☐
- Headache ☐
- Back pain ☐

20. Name any diseases that can cause abortions in cattle.

---

21. Name any diseases of cattle that can affect people.

SECTION C:

22. Fresh cow's milk and home-made cheese are as healthy as packaged fresh milk and home-made cheese from the shop: Yes ☐ No ☐

23. Is it safe to drink milk straight from a cow's udder? Yes ☐ No ☐

24. Handling aborted or stillborn calves with no protection can give you diseases such as brucellosis?
Yes ☐ N ☐ I don't ☐

25. It is safe to handle blood from slaughtered cattle with unprotected hands?
Yes ☐ No ☐ I don't know ☐

26. It is safe to drink blood from slaughtered cattle.
Yes ☐ No ☐ I don't know ☐

27. It is safe to open a cow's uterus using unprotected hands?
Yes ☐ No ☐ I don't know ☐

28. Do you assist cows to give birth at your farm/village?
Yes ☐ No ☐

29. Do you use protected hands (gloves) to assist delivery? Yes ☐ No ☐

30. When my hand (s) are bruised or injured, i:

☐ stop handling and working with meat until they are healed

☐ continue handling and working with meat with uncovered hands

☐ cover hands with gloves and continue handling meat

31. Do you think you are at risk of getting diseases from cattle at the abattoir?
Yes ☐ No ☐

SECTION D:

32. Do you drink raw cow's milk? Yes ☐ No ☐

33. Do you prepare and eat home-made cheeses? Yes ☐ No ☐

34. Do you handle fetuses during slaughter or dressing? Yes ☐ No ☐

If the answer to 34 above is yes, do you wear any protection? _____

35. How do you prepare meat for consumption at home?

Eat raw ☐

Boil thoroughly ☐

Undercook/rare ☐

Roast ☐

36. Which of the following do you eat?

Cattle testicles	Yes ☐	No ☐
Female reproductive parts (uterus, cervix)	Yes ☐	No ☐
Undercooked liver	Yes ☐	No ☐
Uncooked liver	Yes ☐	No ☐
Uncooked blood	Yes ☐	No ☐
Uncooked fore stomachs	Yes ☐	No ☐

37. How frequently does blood splash into your eyes during slaughter, dressing or meat handling?

All the time ☐ Never ☐ Rarely ☐ Sometimes ☐

Appendix 3: Brucellosis survey questionnaire: medical professionals

Please give a response by placing an 'X' in the boxes or by filling in the blank spaces.

Date: _____ **Questionnaire number:** _____

SECTION A:

1. Age: 18-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ >60 ☐
2. Gender: Male ☐ Female ☐

3. Town and Region: _____
4. Education level:
   - Diploma ☐
   - University degree ☐
5. Profession:
   - Medical doctor ☐ Specialization or area of work: _____
   - Nurse ☐ Specialization or area of work (if any): _____
   - Job title: _____
   - Number of years in profession/job: _____

SECTION B:

6. Have you heard of a disease called brucellosis? Yes ☐ No ☐

   6.1 If yes in point 6 above, where did you hear about the disease?
   - During medical training? ☐
   - In the clinic/hospital ☐
   - At a training workshop ☐
   - During interactions with other professionals ☐
   - Other sources (specify) _____

7. How is brucellosis transmitted to humans?

   _____

8. Have you seen a case (s) of brucellosis in people? Yes ☐ No ☐

   If yes:

   8.1 How many cases have you seen in the past year? _____

8.2 Where was the disease observed? Government clinic

Private clinic ☐ Government hospital ☐ Private hospital ☐

8.3 What was the source of the disease in the cases observed?

_____

8.4 What was the history associated with the affected people?

_____

8.5 What were the symptoms in affected people?

_____

8.6 How was the disease confirmed as brucellosis?

_____

8.7 What was the duration of the disease in the observed cases?

_____

8.8 How was the case (s) managed/treated? (Medicines used)

_____

8.9 What was the fate of the patient (s) affected by the disease:

Fully recovered ☐

Developed complications ☐

Died ☐

I don't know ☐

9. What advice would you give to the public with regards to brucellosis prevention?

_____

(a) Have you encountered any cases of unexplained fever in patients? Yes ☐ No ☐

(b) Which diseases are commonly considered as differential diagnosis for unexplained fever in patients?

_____

10. Please specify the zoonotic diseases that you have encountered over the past 10 years and their general trend in the table below (indicate with an 'X'):

	Current trend over the last 10 years in your practice			
Zoonotic disease	**Increasing**	**Decreasing**	**Constant**	**I don't know**

11. Which of the following animal products do you consume?

☐ ☐

Raw milk Yes No

Home-made cheese Yes ☐ No ☐

Home-made yoghurt Yes ☐ No ☐

12. Have you ever assisted cows during delivery? Yes ☐ No ☐

13. Do you think you are at risk of infection with brucellosis? Yes ☐ No ☐

If yes, in what ways are you at risk?

_____

14. How would you rate your level of knowledge of zoonotic diseases?

Fair ☐ Excellent ☐

Poor ☐ Good ☐

15. Do you need training on zoonotic diseases? Yes ☐ No ☐

If yes above, how would you prefer to receive this training?

Online ☐

Face-to-face ☐

Through disease pamphlets ☐ Other: _____

Appendix 4: Informed consent form

FACULTY OF VETERINARY SCIENCE

DEPARTMENT OF VETERINARY TROPICAL DISEASES

INFORMED CONSENT LETTER

Dear Prospective Research Participant

1) INTRODUCTION

You are invited to volunteer for a research study titled **'Knowledge, attitudes and practices related to human and animal brucellosis among occupationally exposed and medical professions'**, a research that forms part of a PhD study at the University of Pretoria.

Principal Investigator: Oscar Madzingira **Telephone number:** +264813593072

Supervisors: Prof. H. van Heerden and Prof. F.O. Fasina

2) PURPOSE AND BENEFITS OF THE STUDY

The study seeks to find out the knowledge, attitudes and risky practices related to brucellosis in communal and cattle farmers; abattoir and butchery workers; nurses and medical doctors. This information can be used to improve brucellosis prevention and control in both the human and animal populations.

3) STUDY PROCEDURES

You are requested to answer the questions on a questionnaire and this will take about 5-15 minutes. Answering the questions is voluntary. Participants may leave out any questions that make them feel uncomfortable or ask the interviewer or PI for clarification. There is no foreseeable physical or other risk associated with participating in this study. All records from this study shall be kept confidential in a secure place. All information collected shall be stored or published in such a way that it is not possible to identify the participants.

4) ETHICS APPROVAL

This Protocol was approved by the Ministry of Health and Social Services (Namibia); Faculty of Veterinary Science (REC056-20) and Faculty of Humanities (HUM026/0620) Research Ethics Committees; and the Animal Ethics Committee (V055-18), University of Pretoria, M35, Onderstepoort, Pretoria, 0110, South Africa, Telephone numbers +27 12 529 8000 / 12 86 100 8387 and written approval was been granted by the committees.

5) CONSENT TO PARTICIPATE IN THIS STUDY

- I confirm that the Principal Investigator has informed me about the nature, purpose, any risks or discomforts, and benefits of the study.
- I have received, read and understood the above written information about the study.
- I was given adequate time to ask questions.
- I am aware that the information obtained in the study, including personal details, shall be handled anonymously at all times, including during reporting of the results.
- I have no objections to participate in this study.
- I understand that I will not be penalised in any way should I wish to discontinue with the study.
- I am participating willingly and do not expect to be paid

_____	_____
Participant's name (Please print)	Date
_____	_____
Participant's signature	Date
_____	_____
Researcher's name (Please print)	Date
_____	_____
Researcher's signature	Date

Appendix 5: Ethical approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Epidemiology and characterisation of Brucella species from bovine tissues in Namibia**
PROJECT NUMBER	**V055-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr O Madzingira**

STUDENT NUMBER (where applicable)	**U_29474257**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES/SAMPLES	**Bovine**
NUMBER OF ANIMALS	**304**
Approval period to use animals for research/testing purposes	**July 2018 – July 2019**
SUPERVISOR	**Prof. H van Heerden**

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

Conditions: The AEC has noted that this project will be completed in a facility outside of South Africa. Since the AEC has not inspected the facility, please note that we cannot comment on the quality of the facility other than what was provided in the study questionnaire

APPROVED (With condition)	Date 28 August 2018
CHAIRMAN: UP Animal Ethics Committee	Signature

Condition: Blood to be collected only after slaughter (post mortem)

S4285-15

Appendix 6: Extension of ethical approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

5 March 2020

Approval Certificate
Annual Renewal (Ext1)

AEC Reference No.:	V055-18
Title:	Epidemiology and characterisation of Brucella species from bovine tissues in Namibia
Researcher:	Dr O Madzingira
Student's Supervisor:	Prof H van Heerden

Dear Dr O Madzingira,
The **Annual Renewal** as supported by documents received between 2019-12-10 and 2020-02-24 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-02-24.

Please note the following about your ethics approval:

1. The use of species is approved:

Species and Samples	Number Available
Cattle	304

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-03-05.
3. Please remember to use your protocol number (V055-18) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8483
Fax +27 12 529 8321
Email aec@up.ac.za
www.up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix 7: Extension of ethical approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

6 July 2020

Approval Certificate
New Application

AEC Reference No.:	REC056-20
Title:	Epidemiology and characterisation of Brucella species from bovine tissues
Researcher:	Dr O Madzingira
Student's Supervisor:	Prof H van Heerden

Dear Dr O Madzingira,

The **New Application** as supported by documents received between 2020-05-23 and 2020-07-03 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-07-03. Please note the following about your ethics approval:

1. The use of species is approved:

Species	**Number**
Cattle	304 (V055-18 previous)
Samples Lymph nodes	 304 (V055-18)
Serum	304 (V055-18)
Spleen	304 (V055-18)
Whole blood EDTA	304 (V055-18)

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-07-06.
3. Please remember to use your protocol number (REC056-20) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8483
Fax +27 12 529 8321
Email aec@up.ac.za
www.up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix 8: REC approval 1

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

Research Ethics Committee 12 June 2020

CONDITIONALLY APPROVAL

Ethics Reference No	**REC056-20**
Protocol Title	**Epidemiology and characterisation of Brucella species from bovine tissues**
Principal Investigator	**Dr O Madzingira**
Supervisors	**Prof H van Heerden**

Dear Dr O Madzingira,

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC056-20) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

NOTES: Conditionally approved. This application was submitted retrospectively; it was conducted with AEC approval only (V055-18).

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

Room 6-6, Arnold Theiler Building
University of Pretoria, Faculty of Veterinary Science
Private Bag X04, Onderstepoort, 0110, South Africa
Tel +27 (0)12 529 8390
Email marie.watson-kriek@up.ac.za
www.up.ac.za

Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa

Appendix 9: REC approval 2

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Fakulteit Geesteswetenskappe
Lefapha la Bomotho

Humanities 100. Since 1919

12 August 2020

Dear Dr O Madzingira

Project Title:	Epidemiology and characterisation of Brucella species from bovine tissues
Researcher:	Dr O Madzingira
Supervisor(s):	Prof H van Heerden
Department:	External department
Reference number:	29474257 (HUM026/0620)
Degree:	Doctoral

I have pleasure in informing you that the above application was **approved** by the Research Ethics Committee on 30 July 2020. Data collection may therefore commence.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should the actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely,

Prof Innocent Pikirayi
Deputy Dean: Postgraduate Studies and Research Ethics
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: PGHumanities@up.ac.za

Appendix 10: Section 20 authorisation

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6 (905)

Dr Oscar Madzingira
Katima Mulilo Campus
University of Namibia
Namibia
Tel: 012 529 8265
E-mail: Henriette.vanheerden@up.ac.za; omadzingira@unam.na

Dear Dr Madzingira,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Your application sent with the email on 21 September 2018 requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The study is approved as per the application form received on 21 September 2018 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HerryG@daff.gov.za;
3. Sampling may only be conducted with relevant authorisation of the Namibian Veterinary Services as indicated and may only originate from farms/ areas that are not under relevant veterinary restriction. Samples may be collected only from Namibian cattle that originate from the areas recognised as FMD, CBPP and PPR free by the OIE. As indicated during the application process, only bovine samples collected at the specified abattoir will be utilised and may include blood and organ samples (lymph-nodes, liver, lung, spleen) as indicated;

4. A veterinary import permit must be obtained prior to importation of specified samples and all conditions stated therein must duly be met. Samples include serum, organ samples and extracted DNA from brucellosis positive cattle to be sent to the DVTD BSL2+ laboratory of the University of Pretoria as specified in the section 20 application process;
5. A veterinary health certificate must accompany the veterinary import permit, whereby the Namibian Veterinary Authority attest that the samples originate from the species and area as specified in the Veterinary Services letter;
6. Samples are to be packed at the Namibian Central Veterinary Laboratory and imported from Namibia via OR Tambo International Airport. All samples must be packaged and transported in accordance with International Air Transport Association (IATA) requirements and the National Road Traffic Act, 1996 (Act No. 93 of 1996);
7. Culturing of tissue samples may be conducted at the BSL2+ laboratory of the DVTD, molecular testing of extracted DNA samples may be conducted at the BSL1 laboratory of the DVTD and samples may be sent to the OVR for biotyping (confirmatory testing) of cultures;
8. All potentially infectious material utilised, collected or generated during the study is to be destroyed at the completion of the study. Records must be kept for five years for auditing purposes;
9. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 approval;
10. A dispensation for the storage of samples as specified during the Section 20 application process is attached.

Title of research/study: "*Epidemiology and characterisation of Brucella species from bovine tissues in Namibia*".

Researcher: Dr Oscar Madzingira

Institution: Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, in collaboration with University of Namibia, Central Veterinary Laboratory of Namibia and the Namibian government.

Our ref Number: 12/11/1/1/6 (905)

Your ref: V055/18

Expiry date: December 2021

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2018 -11- 0 2

SUBJECT: *PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)*