INNOVATIVE APPROACHES TO BRUCELLOSIS DIAGNOSIS

ABDULHAMID SETTENDA LUKAMBAGIRE

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2022

EXTENDED ABSTRACT

Brucellosis is an endemic zoonotic disease of public health importance in many low- and middle-income countries. The main zoonotic species implicated in human infection however are *Brucella melitensis, B. abortus* and *B. suis* infecting over 500,000 humans annually worldwide. Brucellosis also causes production and reproductive losses in livestock globally estimated at about two billion USD annually. Brucellosis is listed as one of six priority zoonoses in Tanzania, where the national One Health strategic plan highlights gaps in current surveillance data needed for the burden estimation of human brucellosis and robust tools for the identification of the species of *Brucella* infecting animals.

This study evaluated the current practices for diagnosing brucellosis based on a retrospective survey of records at health facilities in Arusha Region, northern Tanzania. Patterns of brucellosis testing, brucellosis positivity and test reagent management were evaluated using generalized, linear mixed-effects, regression models, with two main outcomes of interest: brucellosis testing practice and brucellosis test positivity variation compared to the facility type, ownership, ownership, month and year of data collection as explanatory variables. *Brucella* species exposure was estimated using real-time, quantitative polymerase chain reaction assays for the detection and speciation of *Brucella* in blood clots from cattle, goats and sheep in northern Tanzania. The association between animal level characteristics and the results of performance of the Rose Bengal test in this livestock population was evaluated by logistic regression modeling and cross-tabulation. Finally, the performance and costs of serological assays used for human brucellosis in northern Tanzania were evaluated and compared to internationally recommended reference tests.

Significant associations were observed between the probability of brucellosis testing and the year and ownership of the facility where testing was done. The probability of brucellosis testing per month was significantly associated with an interaction between privately owned facilities and the year in which testing was done. The proportion of individuals classified as positive was significantly associated with the type of health facility and the district. Four commercial Febrile *Brucella* agglutination tests (FBATs), sourced from private distributors, were used with variable protocols to test for brucellosis in all the study health facilities. In the detection of *Brucella* spp. from blood clots, fifty-eight (11.6%) of the livestock samples were positive for *Brucella* spp. *Brucella abortus* (31.0%) and *B. melitensis* (38.0%) were detected in samples originating from all three livestock species sampled. However, there was poor agreement (K = 0.102) between the results of the qPCR and the RBT in the livestock population tested. The performance and cost evaluation of frontline serological tests for human brucellosis identified the RBT as the test with highest accuracy (97.7%, CI; 94.7–99.3) and the lowest per-sample cost ($0.69 – $0.79 USD). Comparatively, the often-used FBAT kits had low diagnostic accuracy and higher per-test costs than the RBT.

In addition to the variable protocols for testing, the widespread use of poorly performing FBATs limits the inferences that can be made about the epidemiology of human brucellosis in northern Tanzania. The qPCR assays applied to blood clots detected *Brucella* spp. in cattle, goats and sheep on the ranch in Kagera Region. *Brucella abortus* and *B. melitensis* were each detected in all the samples from cattle, goat and sheep that were positive by the *Brucella* spp. and species-specific qPCR assays. The widely used commercial FBATs performed poorly and cost more than the RBT. The findings from this study highlight areas of focus to achieve improved quality of testing and surveillance of brucellosis at health facilities in Tanzania. Applied to scale, the uniform application of the RBT in health facilities, with quick and accurate reporting of test results and the

exploitation of blood clots for Brucella genus detection are approaches that, applied complementarily to brucellosis diagnosis, could ultimately facilitate the formulation of strategies for prevention and control of this priority disease in many-resource limited settings in sub-Saharan Africa.

DECLARATION

I, **AbdulHamid Settenda Lukambagire**, do hereby declare to the Senate of Sokoine University of Agriculture that this PhD thesis is my own original work, independently done within the period of registration and that it has neither been submitted nor concurrently being submitted in any format to any other university or institution for a higher degree award.

…….. ……..

AbdulHamid Settenda Lukambagire Date

(PhD candidate)

The declaration above is confirmed by;

…….. ……..

Prof. Christopher Jacob Kasanga Date

(Supervisor)

…….. ……..

Prof. Rudovick Reuben Kazwala Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENT

I am highly grateful to Allah (*S.W.T*) for His innumerable blessings bestowed upon me right up to and throughout the course of this PhD.

I acknowledge the Afrique One African Science Partnership for Intervention Research Excellence (AO-ASPIRE) scholarship program (Afrique One-ASPIRE/DEL-15-008, http://afriqueone aspire.org) for the financial and training support throughout the course of this PhD. The Zoonoses in Emerging Livestock Systems (ZELS) program and the University of Glasgow that provided additional facilitation and specific training support pertinent to certain aspects of my PhD studies. The Animal and Plant Health Agency (APHA), brucellosis reference laboratories in the UK, for technical and collaborative assistance in key components of the research work involved in my studies.

I am also grateful for the wonderful supervision, mentorship and critiquing received throughout the course of this PhD: Prof. Rudovick R. Kazwala and Prof. Christopher J. Kasanga, my principal academic supervisors at the College of Veterinary Medicine and Biomedical Sciences, SUA. Prof. Blandina T. Mmbaga at the Kilimanjaro Clinical Research Institute, who has provided mentorship and supervision throughout my postgraduate training and career development. Prof. Gabriel M. Shirima at The Nelson Mandela African Institution of Science and Technology, who provided oversight and supervision both through collaborative research work, within the scholarship consortium and my PhD training. Dr. Jo E.B. Halliday at the University of Glasgow provided hands-on management and supervision of my career development, MSc and PhD studies. I am eternally grateful to you all for the patient and persistent guidance and mentorship offered over the years, despite my stubborn resistance at numerous stages throughout the process.

I also received a lot of critical and insightful assistance from numerous other persons at various collaborating institutions. I cannot mention each and everyone that contributed to

these milestones, but to mention a few critical and representative individuals, at SUA were: Prof. Elliot Phiri, Prof. Robinson Mdegela, Prof. Amandus Muhairwa, Prof. Helena Ngowi, Prof. Hezron Nonga, Prof. Sharadhuli I. Kimera, Prof. Robert Machang'u, Prof. Max Robert, Prof. Claudius Luziga, Dr. Coletha Mathew, Dr. Athuman Lupindu, Dr. Abubakar Hoza, Dr. Ernatus Mkupasi, Dr. Alexanda Mzula and Mr. George Makingi, for their critical evaluation of my PhD work from its conception through to completion. I cannot fairly mention the above individuals, some who also served in respective administrative positions, without acknowledging their dutiful and respective secretaries, administrative assistants and support staff at the Department of Veterinary Medicine and Public Health, the College of Veterinary Medicine and Biomedical Sciences, SUA. My fellowship at SUA was an insightful and educative experience in large part due to your respective and varied input.

To my senior colleagues in the Department of Medical Parasitology and Entomology at the Kilimanjaro Christian Medical University College, who inspired and supported me throughout this journey: Professor Mramba B.A. Nyindo, for your friendship, mentorship and guidance over the years, it has certainly been and exciting honor to work by your side and learn from you. Prof. Franklin Mosha, Prof. Reginald Kavishe, Prof. Johnson Matowo, Dr. Jovin Kitau and Dr. Robert Kaaya I am eternally grateful to you all for the continued presence and assistance at various stages of my training.

Prof. Bassirou Bonfoh, Prof. Sayoki Mfinanga, Dr. Gilbert Fokou, Dr. Richard Yapi at the Centre Suisse de Recherches Scientifiques in Côte d'Ivoire, consortium program fellows and admin; Ms. Zena Babu at SUA, Dr. James M. Akoko, Dr. Ahmed K. Lugelo, Dr. Caroline M. Mburu, Mr. Yoro Staphane-Alain Babo, Drs. Souleymane Traore, Sr. Rosamystica M. Sambu, Raphael R. Mwampashi, Ms. Belinda Mligo and Ms. Hildegalda Colman, all of whom were critical, supportive, and actively engaged in many aspects of fellowship within the Afrique One-ASPIRE program.

I would also like to thank research and project partners at the Kilimanjaro Clinical Research Institute, where the bulk of laboratory work supporting my PhD was conducted and the Duke University Research team at KCRI: Ms. Eliza Kusaga, Ms. Tupokigwe Jana, Mr. Gilbert Shao, Ms. Lucy Sharra, Ms. Prisca Njau, Mr. Frank Micheal and Mr. Francis Karia, your administration, coordination and assistance was well appreciated. Prof. John Crump, Prof. Matthew Rubach, Prof. John Bartlett, Dr. Shama Cash-Goldwasser, Prof. Ireen Kiwelu, Dr. Theonest Ndyetabura, Dr. Tito Kibona, Dr. Kennedy Ngowi, Mr. Davis Kuchaka, Mr. Philoteus Sakasaka, Ms. Saumu Pazia, Mr. Victor Mosha, Mr. Nelson Amani, Ms. Ephrasia Hugo, Ms. Athanasia Maro and Ms. Judith Njau for their valued assistance and cooperation through the years of research work in the Zoonoses Unit at the KCRI Biotechnology laboratory.

Prof. Dan Haydon, Prof. Sarah Cleaveland, Dr. Will deGlanville, Dr. Katharina Kreppel and Dr. Rebecca Bodenham at the University of Glasgow who provided critical collaborative support, training and materials essential to the completion of this PhD, I am eternally grateful for the friendship, support and assistance at various points through this journey. This PhD would have not been completed without the collaborative, technical support and guidance from Dr. Roland Ashford, and Dr. John McGiven at the OIE/FAO Brucellosis Reference Laboratory, Department of Bacteriology, Animal & Plant Health Agency, Surrey, UK.

Last but not least, I would like to thank the management and staff of Endulen Hospital and the Ngorongoro Conservation Area Authority; The Regional Medical Office for Arusha Region and the various District Medical Offices in Arusha for accommodating this study. Finally, the Kagera Sugar Limited Company and the Kitengule Ranch management in Karagwe district for their accommodating support, and readiness to conduct the set of studies documented in this thesis contributed to the successful

collection of data from participants and animals in the respective communities under their management.

DEDICATION

This work and the years of toil leading up to its culmination are dedicated to my parents; my late father, Mr. Ahmed Z. Settenda and my mother, Mrs. Arafah J. Mchinja, both of whom struggled with ill-health throughout my post graduate training, but never failed to extend their dedicated support and encouragement whenever I needed it. May Allah (*S.W.T*) shower His infinite Mercy and Blessings upon you for all that you invested in me.

To my wife and children who patiently bore my frequent absence and shared in the woeful, late nights that permeated our family home throughout this journey. You were a constant reminder and motivation through the toughest of times.

Finally, this work is dedicated to all those close family, friends and colleagues that we started out on this great adventure with and lost along the way. You are all dearly remembered and constantly in our supplications.

TABLE OF CONTENTS

LIST OF PAPERS

This PhD Thesis is organized in a Publishable Manuscript format. It is composed of three journal papers written and formatted in line with publishers' guidelines as summarized below:

PAPER I

AbdulHamid S. Lukambagire, Gabriel M. Shirima, Damas D. Shayo, Coletha Mathew, Richard B. Yapi, Christopher J. Kasanga, Blandina T. Mmbaga, Rudovick R. Kazwala, Jo E.B. Halliday. Brucellosis testing patterns at health facilities in Arusha region, northern Tanzania. PLoS One 2022;17:e0265612.

PAPER II

AbdulHamid S. Lukambagire, James M. Akoko, Coletha Mathew, Rosamystica M. Sambu, Raphael R. Mwampashi, Richard Yapi, Nelson B. Amani, Judith S. Njau, Bassirou Bonfoh, Gabriel M. Shirima, Blandina T. Mmbaga, Christopher J. Kasanga, Robab Katani, Roland T. Ashford, Jo E.B. Halliday, Rudovick R. Kazwala: Direct detection of *Brucella* species in Blood Clots from Livestock in Northern Tanzania (Under Review at *Microorganisms, MDPI*).

PAPER III

Lukambagire A.S, Mendes A.J, Bodenham RF, McGiven AJ, Mkenda NA, Mathew C, Rubach M.P, Sakasaka P, Shayo D.D, Maro V.P, Shirima G.M, Thomas K.M, Kasanga C.J, Kazwala R.R, Halliday J.E.B, Mmbaga B.T. (2021). Performance characteristics and costs of serological tests for brucellosis in a pastoralist community of northern Tanzania. *Scientific Reports (2021) 11:5480*.

LIST OF TABLES

Chapter Two:

Chapter Three:

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

Ab(s)	Antibody (Antibodies)
Ag(s)	Antigen(s)
CDC	Centre for Disease Control and Prevention
cELISA	Competitive Enzyme-Linked Immuno-Sorbent Assay
CVMBS	College of Veterinary Medicine and Biomedical Sciences
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diaminetetraacetic Acid
ELISA	Enzyme-Linked Immuno-Sorbent Assay
LAMP	Loop-Mediated isothermal Amplification PCR
MAT	Microscopic Agglutination Test
Mb	Megabase
MLST	Multi-locus Sequence Typing
NCA	Ngorongoro Conservation Area
NCAA	Ngorongoro Conservation Area Authority
NIMR	National Institute for Medical Research (Tanzania)
PCR	Polymerase Chain Reaction
Prof(s)	Professor(s)
qPCR	Quantitative Polymerase Chain Reaction
RBT	Rose Bengal Test
RNA	Ribonucleic Acid
rpm	Revolutions per minute
spp.	Species
SUA	Sokoine University of Agriculture
TAWIRI	Tanzania Wildlife Research Institute

TZ	Tanzania
USA	United States of America
WHO	World Health Organization
WOAH	World Organization for Animal Health

CHAPTER ONE

1.0 INTRODUCTION

Brucellosis is a zoonotic infection of veterinary and public health importance that is endemic in many low and middle-income countries (LMICs) (Seleem, Boyle, & Sriranganathan, 2010; Skalsky *et al.,* 2008). Over 500 000 new human cases are reported annually, mostly from rural, resource-limited settings (Corbel, 2006; Dean *et al.,* 2012). This number is suspected to be an under-estimation of the true burden of disease (O'Callaghan, 2020). The impact of brucellosis on livestock productivity is not well evaluated either (Roth *et al.,* 2003). However, higher brucellosis prevalences are assumed to have higher productivity losses, approximating two billion USD annually (McDermott *et al.,* 2013). The economic production impacts of brucellosis in livestock species in LMICs have been reported most frequently for cattle (McDermott *et al.,* 2013), with minimal focus on small ruminants. Brucellosis is thus speculated to be the most widespread bacterial zoonosis globally (Al-Dahouk, Sprague, & Neubauer, 2013; Franco *et al.,* 2007). The disease results from infection with bacteria of the genus *Brucella.* To date, up to twelve *Brucella* species have been identified (Jamil *et al.,* 2017). The main species of *Brucella* that are implicated in human infections are *B. melitensis, B. abortus* and *B. suis* (Corbel, 2006). Human exposure to *Brucella* infection mainly occurs through consumption of raw contaminated animal products such as milk and or through contact with aborted materials, placenta or discharges from infected animals *(Solorio-Rivera et al.,* 2007).

Brucellosis is one of six priority zoonoses targeted for control and elimination in Tanzania and many countries in sub-Saharan Africa (SSA). The Tanzania One Health Strategic Plan (2015 – 2020) highlights gains and challenges in the control of brucellosis in both

the public and veterinary health communities (URT, 2015). In Tanzania, brucellosis has been reported on since as early as 1928 (Hammond & Mahlau, 1962) in livestock and 1935 (Wilson, 1936) in humans with numerous reports of studies in both public and veterinary health (Djangwani, Kaindi, Abong, & Njue, 2021; Mengele *et al.*, 2022). The WHO advises the early detection and treatment of brucellosis as the primary intervention for the disease in humans. Active testing and culling of infected individuals coupled with vaccination is the recommended livestock control strategy (Alton, Jones, & Pietz, 1975; WHO, 2020). In areas where the disease has become endemic, with limited means to conduct thorough and extensive testing, the advised diagnosis of human brucellosis should rely on two criteria: clinical presentation with risk of exposure assessment, backed by laboratory diagnosis (Al-Dahouk *et al.*, 2013). However, to date there is no single confirmatory antigen for serology-based testing of acute human brucellosis in Tanzania and much of SSA. Interpretation of results from the current serology-based test regimens requires supporting epidemiological data and risk assessment (CDC, 2017; Corbel, 2006). The national One Health strategy highlights information gaps in these two crucial datasets that are also often missing in many SSA and low to middle income countries (LMICs) (URT, 2018; URT, 2015).

Recently conducted systematic studies evaluating test performance in neighboring countries have demonstrated inconsistencies in the epidemiological and diagnostic data generated from the currently available serological tests in use (deGlanville *et al.*, 2017; Alumasa *et al.*, 2021). In light of these findings, there is a need for well-controlled and systematic studies to assess the diagnostic tools available and in use for brucellosis in Tanzania. Further adoption of novel approaches are required to address the clinical and population-specific challenges presented by inadequately performing serological tests (URT, 2018).

A number of robust antigen-based, serology diagnostic tests aimed at the detection of circulating host antibody to specific epitopes of *Brucella* bacteria have been developed (McGiven *et al.,* 2015). The specific challenges presented by human brucellosis require an informative test to aid the clinical distinction between acute illness, relapse after treatment and chronic brucellosis, as well as sero-positivity due to previous exposure to *Brucella* in absence of the disease (Franco *et al.,* 2007; McGiven *et al.,* 2015; Díaz *et al.*, 2011). This poor distinction, coupled with the low quality of antigens used in preparation of test reagents, is thought to be a driving factor behind the poor performance of currently available rapid sero-diagnostic tests in application to human brucellosis (Al Dahouk & Nöckler, 2011; de Glanville *et al.,* 2017; Moreno, Blasco, & Moriyón, 2022).

The development and advancement of molecular-based specific tests has significantly improved the ease and precision of detecting circulating *Brucella* DNA to the species and biovar level (Bricker & Halling, 1994; Pérez-Sancho *et al.,* 2013). The traditional culture techniques often present added risk of infection to laboratory personnel, are difficult and time-consuming to perform and require minimum safety environment standards that are often lacking in resource-limited settings (Al-Dahouk *et al.,* 2013; Ducrotoy & Bardosh, 2017). The simplification of such high performance, robust tests would present significant advances in brucellosis diagnostics, providing a much needed advantage in the control and prevention programs designed for LMICs (Ducrotoy *et al.*, 2017; O'Callaghan, 2020).

In Tanzania to date, the recommended test guidelines for human brucellosis include; the Rose Bengal Test (RBT) as the first line serology-based screening test followed by the mercaptoethanol test for confirmation (URT, 2018). Although culture and isolation of the infectious bacteria is the recommended confirmatory test for brucellosis, there are few testing facilities that have this capacity in place. The bacteriological confirmation of

isolated *Brucella* or identification by PCR-based molecular tests in positive cases is an assay that is not yet available in any treatment facilities, but remains the confirmatory gold standard for clinical diagnosis of brucellosis. The latter two test options are often used by research projects and have contributed to much of the reported disease epidemiological data available in the country (URT, 2015; CDC, 2017).

A crucial step in the standardization of test systems is a controlled evaluation of the tests and methods in use (OIE, 2019). Assuming consistent use of standard, recommended reagents, the performance of assays may still vary considerably in different locations, when conducted by different persons and under different environmental conditions. The optimization of test assays under local conditions is therefore recommended as a first step to standardizing result generation (OIE, 2019). This study developed and evaluated novel approaches to diagnosis based on previously established systems and assays.

1.1 Problem Statement and Justification

Brucellosis is one of the world's most widespread bacterial zoonoses and has been identified as one of six priority zoonotic diseases in Tanzania (URT, 2015; WHO, 2020). The transmission dynamics of brucellosis place the disease burden higher among rural, pastoral and agro-pastoral communities with large flocks and high-risk contact between people and animals. Implementation of prevention and control measures is complicated by several factors. Firstly, Tanzania has a complex, mostly rural-based livestock sector ranging from smallholders, agro-pastoral mixed farming to extensive pastoral systems. These systems however have limited access to high precision, quality testing for brucellosis both among the human and animal populations. Brucellosis has no definitive disease presentation, making clinical diagnosis of both humans and animals difficult, with illness often being misdiagnosed as one of myriad other tropical infectious diseases. Some

productivity and reproductive complications could raise suspicion of infection within a herd, but such symptoms may also be caused by infection with a variety of other pathogens. In addition, brucellosis has hitherto been a neglected zoonosis with low priority as a cause of illness. This has led to brucellosis rarely being prioritized by healthcare providers, allocating insufficient time, focus and resources towards detection and mitigation of the disease. Poorly regulated testing practices and resources are often applied in resource limited rural settings, typical of the Tanzanian communities most burdened by brucellosis.

In Tanzania and much of SSA to date, despite national and regional brucellosis diagnostic and control policies, there remains an inaccurate picture of the actual disease burden in humans as well as the species-specific characterization of infections in livestock. These challenges are in part due to the poor implementation of the policies in place, as a result of poor access to diagnostic tools. These deficiencies directly impact on the estimation of brucellosis burden in Tanzania and other developing countries where the disease is endemic and neglected. Under reporting, and in some scenarios, over-reporting of brucellosis in humans and animals leads to a misrepresentation of the scale of the problem and consequently, misappropriation of investment in the research and control approaches required to mitigate impacts attributable to this disease. Availability of more robust diagnostic tools and quality data facilitates an improved understanding of the burden of brucellosis in human and livestock populations respectively. This in turn informs options for future control strategies, proficiency testing guidelines and policy advisement on the range and application of tests available. The study aimed to understand the prevailing practices for the procurement, test performance and result utilization in the diagnosis of human brucellosis in health facilities in Arusha Region, northern Tanzania. Real-time qPCR assays were further applied to in livestock samples for the detection and

speciation of *Brucella.* Finally, compilation of samples and analysis of robust datasets from studies conducted in high-risk populations were used to assess the performance and cost implications of the current and proposed serology-based test options for human brucellosis in Tanzania. This approach aimed to quantify the performance of currently used test systems for diagnosis, management and control policies for brucellosis in Tanzania, providing practical options for their potential improvement. Lessons learned from this study can also be applied to the East African region and other endemic settings in SSA.

1.2 Research Questions

1. What are the procurement, test performance and reporting patterns of brucellosis at health facilities in Arusha Region, northern Tanzania?
2. What is the appropriate PCR-based assay for the real-time identification of infectious *Brucella* species in low-resource settings?
3. What are the optimal performance characteristics of the novel test assays developed under laboratory and field conditions?

1.3 Objectives of the study

1.3.1 Main Objective

To deploy and evaluate simple, novel and real-time diagnostic tests for brucellosis in human and livestock populations in resource-limited areas of Tanzania

1.3.2 Specific Objectives

- To determine the practices of test purchase, performance and reporting of human brucellosis at health facilities in northern Tanzania

- To determine the appropriate real-time, species-specific test assay for brucellosis in low resource areas in Tanzania
- To evaluate the performance of serology and molecular based test options for brucellosis in Tanzania under laboratory and field conditions

1.4 Organization of the Thesis

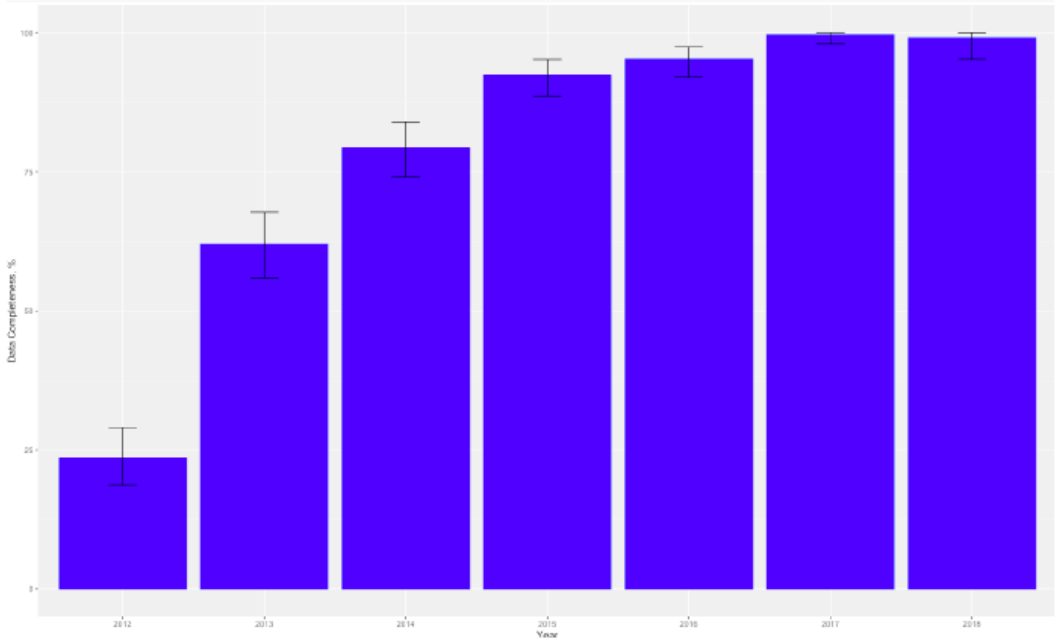
This PhD Thesis is organized such that Chapter One contains: the introduction, problem statement and justification, research questions, overall objective and specific objectives. Chapters Two through Four comprise Papers I, II and III, respectively as submitted for publication in peer-reviewed, scientific journals. The results related to each specific objective of this thesis are presented and discussed in: Journal Paper I (*PLoS One 2022;17:e0265612*) detailing the findings of a survey of health facility diagnostic and reporting practices of brucellosis in Arusha Region, northern Tanzania. Manuscript II (under review at *Microorganisms, MDPI*) describes the application of real-time molecular assays for the direct detection and speciation of *Brucella* DNA in livestock blood clots from Tanzania. Journal Paper III (*Scientific Reports 2021;11:5480*) details the performance and cost evaluation of seven index serological tests used for brucellosis in northern Tanzania. Chapter Five draws overall conclusions and puts forward recommendations emanating from this study in relation to the main study objectives. Attachments, appendices and references for the respective journal papers and the manuscripts are attached at the end of the papers or manuscripts chapters. The thesis ends with the reference list for Chapters One and Five.

CHAPTER TWO

2.0 BRUCELLOSIS TESTING PATTERNS AT HEALTH FACILITIES IN ARUSHA REGION, NORTHERN TANZANIA

Chapter 2: Paper Supplementary Information

Supplementary Information: Brucellosis testing patterns at health facilities in Arusha region, northern Tanzania (Plos One: https://doi.org/10.1371/journal.pone.0265612)



S1 Fig. Graph showing data completeness for the 23 facilities contributing data over the seven years of the data collection period. Data completeness is calculated assuming that all facilities operated for the full study period and accounts for the fact that only five months of data were included for 2018.

S1 Table: Facility characteristics for the study period from January 2011 to May 2018 inclusive (n = 24 facilities); CI- Confidence interval

Variable	Category	n/N	Percentage (95% CI)
Ownership	Faith-based organization	8/24	33.3 (15.6 – 55.3)
	Government	13/24	54.2 (32.8 – 74.4)
	Private	3/24	12.5 (2.7 – 32.4)
Clinical diagnosis done	Yes	5/24	20.8 (7.1 – 42.2)
	No	19/24	79.2 (57.8 – 92.9)
Brucellosis testing done	Yes	21/24	87.5 (67.6 – 97.3)
	No	3/24	12.5 (7.1 42.2)
Reason for not testing	No reagent	14/21	66.7 (43.0 – 85.4)
	No reagent controls	4/21	19.0 (5.4 – 42.0)
	Don't report brucellosis	3/3	100 (0.3 – 100)
Brucellosis reagent currently used for testing	Eurocell	17/21	81.0 (58.1 – 96.0)
	Fortress	9/21	42.9 (21.8 – 66.0)
	Genuine Biosystem	7/21	33.3 (14.6 – 57.0)
	Arkray	4/21	19.0 (5.4 – 42.0)
	Rose Bengal Test	0/21	-
Brucellosis reagent reported previous use	Eurocell	10/21	47.6 (25.7 – 70.2)
	Fortress	8/21	38.1 (18.1 – 61.6)
	Genuine Biosystem	8/21	38.1 (18.1 – 61.6)
	Arkray	4/21	19.0 (5.4 – 42.0)
	Rose Bengal Test	1/21	4.8 (0.1 – 23.8)
Reagent supplier	Supplier 1 (Arusha)	17/21	81.0 (58.1 – 96.0)
	Supplier 2 (Arusha)	7/21	33.3 (14.6 – 57.0)

Reagent purchase cost in TZS	30,000	12/21	57.1 (34.0 – 78.2)
	35,000	1/21	4.8 (0.1 – 23.8)
	45,000	10/21	47.6 (25.7 – 70.2)
	50,000	8/21	38.1 (18.1 – 61.6)
	60,000	4/21	19.0 (5.4 – 42.0)
	65,000	3/21	14.3 (3.0 – 36.3)
Test cost to patient in TZS (Cat)	2000 – 3000	12/21	57.1 (34.0 – 78.2)
	3000 – 5000	11/21	52.4 (29.8 – 74.3)
	5000 – 10000	1/21	4.8 (0.1 – 23.8)
Do you se controls in test runs	Yes	5/21	23.8 (8.2 – 47.2)
	No	16/21	76.2 (52.8 – 91.8)
Control material used	Kit provided	3/5	60.0 (14.7 – 94.7)
	Positive serum	2/5	40.0 (5.3 – 85.3)
	Both	2/5	40.0 (5.3 – 85.3)
Performed QA for each run	Yes	5/21	23.8 (8.2 – 47.2)
	No	16/21	76.2 (52.8 – 91.8)
Run serial dilutions	Yes	1/21	4.8 (0.1 – 23.8)
	No	20/21	95.2 (76.2 – 99.9)
Test result reported	Pos or Neg	15/21	71.4 (47.8 – 88.7)
	Pos (*B. abortus, B. melitensis*) or Neg	5/21	23.8 (8.2 – 47.2)
	Titres	1/21	4.8 (0.1 – 23.8)
Any test re-run	Yes	8/21	38.1 (18.1 – 61.6)
	No	13/21	61.9 (38.4 – 81.9)
Reason for rerun test	Result verification	5/8	62.5 (24.5 – 91.5)
	Confirm recovery	3/8	37.5 (8.5 – 75.5)

CHAPTER THREE

3.0 DIRECT DETECTION OF BRUCELLA SPECIES IN BLOOD CLOTS FROM LIVESTOCK IN NORTHERN TANZANIA

Chapter 3: Paper Supplementary Information

Table S1: Summary of the primer and probe sequences for the *Brucella* spp. and species-specific qPCR targets.

Assay	Target	Forward Primer	Reverse Primer	Probe	Ref
Brucella spp.	IS*711*	3′-GGCCTA CCGCTG CGAAT-5′	5′-TTGCGG ACAGTC ACCATA ATG-3′	FAM-AAGCCAACA CCC GGC-MGBNFQ	[35]
	bcsp31	3′-GCTCGGTTGCCAA TATCAATGC-5′	5′-GGGTAAAGCGTCG CCAGAAG-3′	6FAM-AAATCTTCCACC TTGCCCTTGCCATCA-BH Q1	[3]
Brucella speciation	*alk B*	3′-GCGGCTTTTCTAT CACGGTATTC-5′	5′-CATGCGCTATGATC TGGTTACG-3′	HEX-CGCTCATGCTCGC CAGACTTCAATG-BHQ1	[46]
	BMEI1162	3′-AACAAGCGGCAC CCCTAAAA-5′	5′-CATGCGCTATGATC TGGTTACG-3′	TexasRed-CAGGAGTGTT TCGGCTCAGAATAATCC ACA-BHQ2	[46]

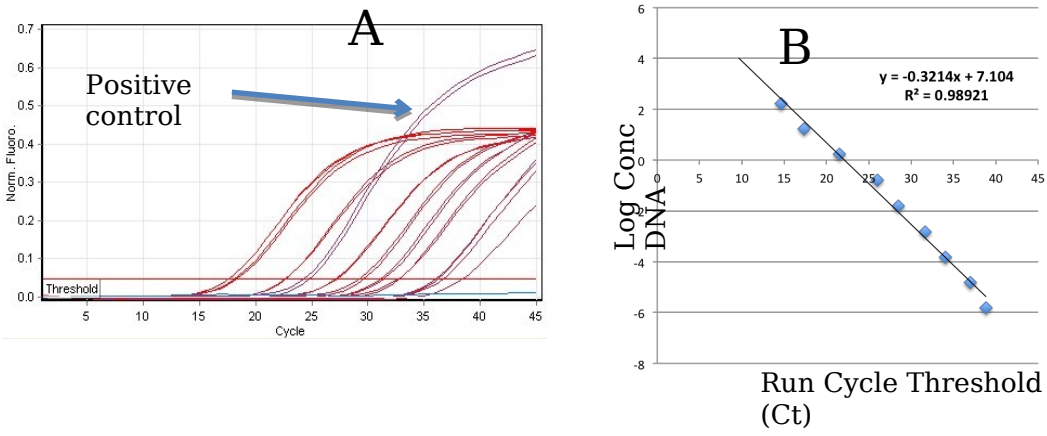


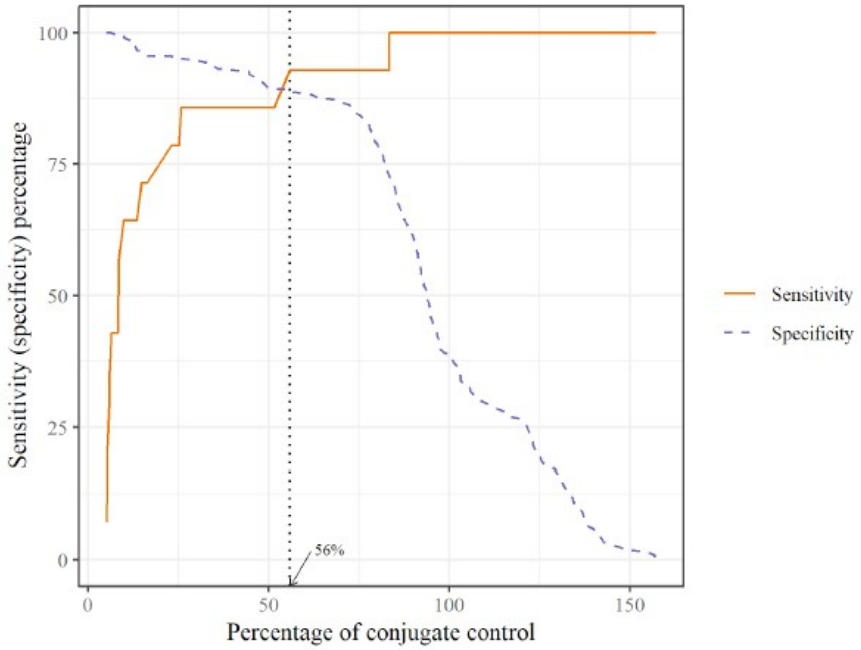
Fig 2, SI: Plot of the amplification curve for serially diluted spiked clot extracts detecting IS711 (A) and the standard curve generated from the serially diluted spiking experiments (B).

CHAPTER FOUR

4.0 PERFORMANCE CHARACTERISTICS AND COSTS OF SEROLOGICAL TESTS FOR BRUCELLOSIS IN A PASTORALIST COMMUNITY OF NORTHERN TANZANIA

Chapter 4 APPENDIX: Paper Supplementary Information

Supplementary materials



S1. Two-Graphs Receiving Operating Characteristics. The black dotted line indicates the threshold used as cut-off for classification. At this value, the sum of sensitivity and specific is maximized.

S2. Pricing sources and details assumed for all component costs in cost estimation (currency: USD): Price assumes purchase in Tanzania Shillings (conversion rate to 1 $ (USD) on 11/07/2019 from Central Bank of Tanzania at Spot Buying: 2,277.69 TZS), except for items purchased from APHA Scientific as the currency was the pound sterling (conversion rate to 1 £ (GBP) on 11/07/2019 from Central Bank of Tanzania at Spot Buying: 2,851.21 TZS); U/PS: units used per patient sample; U/QC: number of units for QC per batch (run); T/K: tests per kit; YL: 10 years of lifespan; MC/Y: maintenance cost per year in USD; PU/D: percentage use for method per day; RBT: the Rose Bengal test (both RBT 1:2 and RBT 1:8 protocols); cELISA: competitive enzyme-linked immunosorbent assay.

Cost category	Item	Reference / manufacturer	Price	Details		
Reagents and consumables				**U/PS**	**U/QC**	**Other details**
	Eurocell kit	56/EME/1210/VER-01	7.03	1	2	100 T/K
	Fortress kit	FEBAMP-FEBRILE v3	14.05	1	2	100 T/K; controls included
	Arkray kit	15SA402-05/3-05	13.17	1	2	100 T/K; controls included
	Amitech kit	Amitech Diagnostics Inc.	13.17	1	2	100 T/K
	RBT antigen	APHA Scientific	316.71	1	2	4,000 T/K
	cELISA kit	APHA Scientific	264.13	1	2	400 T/K; controls included
	Eurocell controls	56/EME/1210/VER-01	7.03	0	1	10 T/K
	Amitech controls	Amitech Diagnostics Inc.	5.71	0	1	10 T/K
	RBT controls	APHA Scientific (RAB1003 and RAB 0701)	65.09	0	1	4,000 T/K
	Gloves	Fisher Scientific	3.29	2	2	pack of 100 units
	Tips	ABS	25.07	2 (except 4 for RBT 1:8 and 7 for cELISA)	2 (except 7 for cELISA)	pack of 1,000 units of 1,000 µL without filter
	Stirrer	Local	0.22	1 (except 0 for cELISA)	1 (except 0 for cELISA)	pack of 100 toothpicks
	Saline	Local	1.54	5 (except 2 for RBT 1:8, and 0 for RBT 1:2, Fortress and cELISA)	1 (except 0 for RBT 1:2, Fortress and cELISA)	1L of clinical grade saline; 1 unit = 0.05 ml
	Tile	Local	1.01	0.001	0.001	1 unit = 1 x 0.5 ft tile
	Disposable gown	Fischer Scientific	6.59	0.1	0.1	pack of 25 units
	Surface disinfectant wipes	Local	7.50	0.1	0.1	pack of 200 units

S3: Values and distributions assumed for the probabilistic sensitivity analysis. Average time required to run a batch of (ATRB) Eurocell, Fortress, Arkray and Amitech: 60 minutes; ATRB RBT(1:2): 30 minutes; ATRB RBT(1:8): 35 minutes; ATRB cELISA: 120 minutes; Laboratory average working hours per day, days per year, weeks per year, runs per week, and tests per year: 8, 312, 52, 6, 1560; RBT: the Rose Bengal test (both RBT 1:2 and RBT 1:8 protocols); cELISA: competitive enzyme-linked immunosorbent assay.

Parameter	Value / distribution	Units
Average time required to run a batch of (ATRB)	Uniform (50,70)	Minutes
ATRB RBT (1:2)	Uniform (25,35)	Minutes
ATRB RBT (1:8)	ATRB RBT (1:2) + 5	Minutes
ATRB cELISA	Uniform (110,130)	Minutes
Laboratory average working hours per day	Uniform (6,10)	Hours
Laboratory average working days per week	Uniform (5,7)	Days
Laboratory average working weeks per year	Fixed (52)	Weeks
Testing schedule (number of runs per week)	Uniform (5,7)	N/A
Annual salary	Gamma (7902,2)	USD
Rent / year	Gamma (1250,0.25)	USD
Building maintenance / year	Gamma (62.5,0.25)	USD
Water / year	Gamma (110,1)	USD
Electricity / year	Gamma (110,1)	USD
Internet and telephone / year	Gamma (25,0.5)	USD
Other costs / year	Gamma (25,0.5)	USD
Annual proficiency testing panel	Gamma (50,0.5)	USD
Annual audit	Gamma (175,0.5)	Units

S4. Pairwise comparison of sensitivity (top / right; blue cells) and specificity (bottom / left; red cells) estimates from each combination of index tests (n=218). All pairwise metrics were tested by differences between proportions. RBT - the Rose Bengal test (both RBT 1:2 and RBT 1:8 protocols); cELISA - competitive enzyme-linked immunosorbent assay. The statistical significance of the test of pairwise differences are shown as follows: *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001.

		Test						
		RBT 1:2	RBT 1:8	Amitech	Arkray	Eurocell	Fortress	cELISA
Test	RBT 1:2			*			*	
	RBT 1:8						*	
	Amitech	***	***					**
	Arkray	***	***					
	Eurocell	***	***	**	*			*
	Fortress	***	***		*	***		**
	cELISA	**	***	***	***	***	***	

Sensitivity Specificity

S5. Index and reference test results for each sample. RBT - the Rose Bengal test (both RBT 1:2 and RBT 1:8 protocols); cELISA - competitive enzyme-linked immunosorbent assay.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study provides evidence for revisiting the diagnostic options available in the country and introduction of novel approaches to support nation-wide, uniform diagnosis and surveillance of brucellosis within a national One Health framework. The prioritization of brucellosis, among other emerging and re-emerging zoonoses, has been a critical step in efforts towards its control and elimination in Tanzania and much of SSA. The availability of high-quality, evidence-based data to support the national One Health strategy thus remains a critical cornerstone in the implementation of large-scale, control strategies.

First, the study evaluated the practices and patterns in diagnosis of brucellosis at health facilities in Arusha Region, northern Tanzania. The poorly performing FBAT kits were the mainstay of serological testing, with no use of recommended reagents or standardized protocols. Brucellosis testing was variable in the different facility types and locations, with a significant proportion of tested patients classified as positive but not captured within the prevailing health information and reporting systems. These findings highlight the urgent need to move to standard, recommended and centrally sourced test reagents, using uniform protocols to achieve effective national surveillance and control programs. The study of health facility records and reporting practices also highlight the need for a more systematic and coordinated reporting of brucellosis within the current health information and surveillance framework.

The real-time qPCR assays were applied to blood clot extracts for the detection of *Brucella* spp., a novel approach that has scarcely been reported in the literature. This

approach could potentially minimise the cost of surveillance programs by maximising the use of collected samples to test for brucellosis alongside other routinely monitored diseases. The species-specific assays detected the presence of zoonotic *B. abortus* and *B. melitensis* species in a large proportion of all the livestock species sampled. Although there was very poor agreement between the qPCR assay and the RBT results, the study findings highlight the benefits of enriching serological surveillance data on brucellosis with the real-time molecular detection and speciation observed in this livestock population. The study thus provides practical data for the deployment and exploitation of real-time molecular assays to significantly improve the diagnostic and surveillance platforms for brucellosis in animal populations.

Lastly, the performance and cost evaluation of multiple serological tests used in health facilities in northern Tanzania demonstrated the higher test accuracy and lower cost of the RBT, including a revised testing protocol, as compared to the commercial FBAT kits currently in use across the country. These findings provide robust data and evidence for the likely benefits of implementing coordinated, nationwide use of the RBT in health facilities and argue against the continued use of the poorly performing, commercial FBAT kits. Although not among the list of recommended confirmatory test options, the cELISA kit had high test accuracy and favorable per-sample cost when applied as a secondary test as compared to its use a frontline test for human brucellosis. This study highlights the poor performance and unfavorable cost per sample of the common FBAT kits used in Tanzanian health facilities, with potential benefits of replacement with the RBT conducted under an improved, uniform protocol for health facility use.

5.2 Recommendations

1. There is a need for the uniform application of testing protocols using recommended, tests for a clear understanding of the current burden of human brucellosis in Tanzania. The study findings provide critical evidence and the need for policy advocation for the discontinued use of the commercial FBAT kits, and implementation of the RBT as recently proposed (Lukambagire et al., 2021).
2. As a One Health priority zoonosis, there is a need for uniform testing and surveillance systems for brucellosis to support easier coordination and interpretation of test results.
3. The current challenges within the healthcare system such as procurement practices, centralised sourcing of test reagents, lack of standardised case definitions and management of brucellosis still need to be addressed on a national level, before the benefits of the above recommendations can be fully realised.
4. In addition to improved testing and clear case definition, comprehensive and real-time reporting systems that capture cases of brucellosis at health facilities are still required and have already been recommended in the current national guidelines (URT, 2018; Mligo *et al.,* 2021).
5. The real-time qPCR assays applied to novel sample types e.g. blood clots could significantly cut down on the hazards, cost and delays in detection and characterization of *Brucella* within a routine and support framework for national-level surveillance systems.
6. Further investigation of possible *Brucella* strain variations that could impact analytical performance of the molecular assays is still needed in much of SSA.

CHAPTER SIX

6.0 REFERENCES

Al-Dahouk, S., Sprague, L. D., & Neubauer, H. (2013). New developments in the diagnostic procedures for zoonotic brucellosis in humans. *Scientific and Technical Review of the Office International Des Epizooties (Paris)*, *32*(1), 177–188. https://doi.org/DOI: 10.20506/rst.32.1.2204

Al Dahouk, S., & Nöckler, K. (2011). Implications of laboratory diagnosis on brucellosis therapy. *Expert Review of Anti-Infective Therapy*, *9*(7), 833–845. https://doi.org/10.1586/eri.11.55

Alton, G. G., Jones, L. M., & Pietz, D. E. (1975). Laboratory techniques in brucellosis. *Monograph Series. World Health Organization*, pp. 1–163. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/38676/WHO_MONO_55_part2.pdf

Alumasa, L., Thomas, F. L., Amanya, F., Njoroge, S. M., Moriyón, I., Makhandia, J., … Falzon, L. C. (2021). Hospital-based evidence on cost-effectiveness of brucellosis diagnostic tests and treatment in Kenyan hospitals. *PLOS Neglected Tropical Diseases*, *891*, 1–19. https://doi.org/10.1371/journal.pntd.0008977

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. https://doi.org/10.1128/jcm.32.11.2660-2666.1994

Center for Disease Control and Prevention. (2017). *Brucellosis Reference Guide: Exposures, Testing and Prevention*. pp 1–35. Retrieved from https://www.cdc.gov/brucellosis/pdf/brucellosi-reference-guide.pdf

Corbel, M. M. J. (2006). Brucellosis in humans and animals Brucellosis in humans and animals. *WHO Library Catalogue in Publication Data*, *2006*, 1–88. https://doi.org/10.2105/AJPH.30.3.299

de Glanville, W. A., Conde-Álvarez, R., Moriyón, I., Njeru, J., Díaz, R., Cook, E. A. J., & Fèvre, E. M. (2017). Poor performance of the rapid test for human brucellosis in health facilities in Kenya. *PLoS Neglected Tropical Diseases*, *11*(4), 1–15. https://doi.org/10.1371/journal.pntd.0005508

Dean, A. S., Crump, L., Greter, H., Hattendorf, J., Schelling, E., & Zinsstag, J. (2012). Clinical Manifestations of Human Brucellosis: A Systematic Review and Meta-Analysis. *PLoS Neglected Tropical Diseases*, *6*(12). https://doi.org/10.1371/journal.pntd.0001929

Díaz, R., Casanova, A., Ariza, J., & Moriyón, I. (2011). The rose Bengal test in human brucellosis: A neglected test for the diagnosis of a neglected disease. *PLoS Neglected Tropical Diseases*, *5*(4), 1–7. https://doi.org/10.1371/journal.pntd.0000950

Djangwani, J., Kaindi, D. W. M., Abong, G. O., & Njue, L. G. (2021). Brucellosis : Prevalence with reference to East African community countries – A rapid review. *Veterinary Medicine and Science*, *3*(425), 1–17. https://doi.org/10.1002/vms3.425

Ducrotoy, M., Bertu, W. J., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A. M., & Moriyón, I. (2017). Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta Tropica*, *165*, 179–193. https://doi.org/10.1016/j.actatropica.2015.10.023

Ducrotoy, M. J., & Bardosh, K. L. (2017). How do you get the Rose Bengal Test at the point-of-care to diagnose brucellosis in Africa? The importance of a systems approach. *Acta Tropica*, *165*, 33–39. https://doi.org/10.1016/j.actatropica.2016.10.004

Franco, M. P., Mulder, M., Gilman, R. H., & Smits, H. L. (2007). Human brucellosis. *The Lancet Infectious Diseases*, *7*, 775–786. https://doi.org/10.1016/S1473-3099(07)70286-4

Hammond, J. A., & Mahlau, E. A. (1962). A brucellosis survey of the Western areas of Tanganyika. *Bulletin of Epizootic Disease in Africa*, *10*, 511–516. Retrieved from https://agris.fao.org/agris-search/search.do?recordID=XF2016005026

Jamil, T., Melzer, F., Njeru, J., El-Adawy, H., Neubauer, H., & Wareth, G. (2017). Brucella abortus: Current Research and Future Trends. *Current Clinical Microbiology Reports*, *4*(1), 1–10. https://doi.org/10.1007/s40588-017-0052-z

Lukambagire, A., Mligo, J., Mwakapeje, E., Mathew, C., Shirima, G., Karimuribo, E., … Mmbaga, B. (2021). Brucellosis Diagnosis: Performance, Reporting and Costs of Frontline Tests. *Tanzania Public Health Bulletin*, *1*(6), 27–31. Retrieved from https://mcusercontent.com/00fbd4ff91f363531ad64315d/files/b86d2e60-2e2f-3e6e-b6c8-8bd91a2297bc/Lukambagire_et_al_Brucellosis_Diagnosis_TPHB_Issue_6_27_31.01.pdf

McDermott, J. J., Grace, D., & Zinsstag, J. (2013). Economics of brucellosis impact and control in low-income countries. *Sci. Tech. Rev. Off. Int. des Epizoot.*, *32*, 249–261.

McGiven, J., Howells, L., Duncombe, L., Stack, J., Ganesh, N. V., Guiard, J., & Bundle, D. R. (2015). Improved serodiagnosis of bovine brucellosis by novel synthetic oligosaccharide antigens representing the capping M epitope elements of Brucella O-Polysaccharide. *Journal of Clinical Microbiology*, *53*(4), 1204–1210. https://doi.org/10.1128/JCM.03185-14

Mligo, J. B., Sindato, C., Yapi, B. R., Mathew, C., Mkupasi, E., Kazwala, R. R., & Karimuribo, E. D. (2021). Frontline health workers' awareness and use of E-based technology to enhance reporting of human brucellosis in rural setting in Tanzania. *BMC One Health Outlook*, *56*(5). https://doi.org/doi: 10.1186/s42522-021-00056-5

Moreno, E., Blasco, J., & Moriyón, I. (2022). Facing the Human and Animal Brucellosis Conundrums : The Forgotten Lessons. *Microorganisms*, *10*(942), 1–30. https://doi.org/https://doi.org/10.3390/ microorganisms10050942

O'Callaghan, D. (2020). Human brucellosis: Recent advances and future challenges. *Infectious Diseases of Poverty*, *9*(1), 9–10. https://doi.org/10.1186/s40249-020-00715-1

OIE. (2019). Chapter 1.1.2. - Principles and methods of validation of diagnostic assays for infectious diseases. In *OIE Manual of Diagnostic Tests for Aquatic Animals* (2019th ed., pp. 1–18). Retrieved from http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.6.00_INTRODUCTION.pdf

Pérez-Sancho, M., García-Seco, T., Arrogante, L., García, N., Martínez, I., Diez-Guerrier, A., & Álvarez, J. (2013). Development and evaluation of an IS711-based loop mediated isothermal amplification method (LAMP) for detection of Brucella spp. on clinical samples. *Research in Veterinary Science*, *95*(2), 489–494. https://doi.org/10.1016/j.rvsc.2013.05.002

Roth, F., Zinsstag, J., Orkhon, D., Chimed-Ochir, G., Hutton, G., Cosivi, O., Carrin, G., & Otte, J. (2003). Human health benefits from livestock vaccination for brucellosis: Case study. Bull. World Health Organ. 81, 867–876. https://doi.org/10.1590/S0042-96862003001200005

Seleem, M. N., Boyle, S. M., & Sriranganathan, N. (2010). Brucellosis: A re-emerging zoonosis. *Veterinary Microbiology*, *140*(3–4), 392–398. https://doi.org/10.1016/j.vetmic.2009.06.021

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. *Bmj*, *336*(7646), 701–704. https://doi.org/10.1136/bmj.39497.500903.25

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*(3–4), 282–290. https://doi.org/10.1016/j.prevetmed.2007.05.024

The United Republic of Tanzania (URT). (2018). National Strategy for Prevention and Control of Brucellosis in Humans and Animals 2018-2023. In *Prime Ministers Office* (Vol. 56). Retrieved from https://www.mifugouvuvi.go.tz/uploads/ publications/ sw1602245043-National Strategy On Prevention And Control Of Brucellosis In Humans And Animals.pdf

United Republic of Tanzania (URT). (2018). Guidelines for Surveillance of Prioritized Zoonotic Diseases for Human and Animal Health in the United Republic of Tanzania. In *Government Report* (Vol. 1). Dar es Salaam.

United Republic of Tanzania (URT), United States Department of Defense (DoD), Defense Threat Reduction Agency (DTRA), Cooperative Threat Reduction (CTR), & Cooperative Biological Engagement Program (CBEP). (2015). *The United Republic of Tanzania One Health Strategic Plan 2015-2020*. Retrieved from http://www.tzdpg.or.tz/fileadmin/documents/dpg_internal/dpg_working_groups_clusters/cluster_2/health/Key_Sector_Documents/ Tanzania_Key_Health_Documents/ FINAL_URT_One_Health_Strategy_Plan_20151021.pdf

Wilson, D. E. (1936). Human brucellosis in Tanganyika territory. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *XXIX*(4), 363–368.

World Health Organization (WHO). (2020). Brucellosis. WHO Fact Sheet on Brucellosis website: https://www.who.int/news-room/fact-sheets/detail/brucellosis. Retrieved May 20, 2022, from

APPENDICES

Appendix 1: Tanzania Public Health Bulletin, Policy Brief

Health news bulletin from the Tanzania's Ministry of Health

---

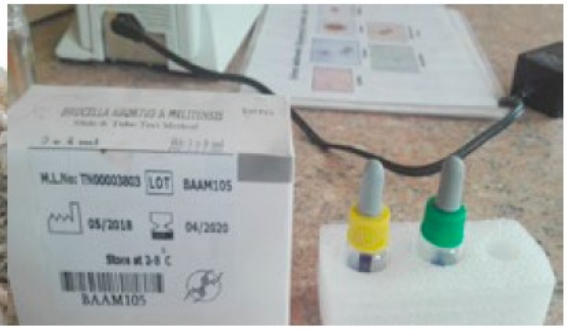
volume1, No.1(Issue 6)

Brucellosis Diagnosis: Performance, Reporting and Costs of Frontline Tests

Lukambagire AS[1,2], Mligo JB1, Mwakapeje E[3], Mathew C[1], Shirima G4, Karimuribo ED[3], Kazwala R[3], Mmbaga BT[2,5]

Brucellosis Diagnosis: Performance, Reporting and Costs of Frontline Tests HERE

Brucellosis Diagnosis: Performance, Reporting and Costs of Frontline Tests



Lukambagire AS[1,2], Mligo JB1, Mwakapeje E[3], Mathew C[1], Shirima G4, Karimuribo ED[1], Kazwala R[1], Mmbaga BT[2,5]

KEY MESSAGE

- Many rural healthcare facilities lack diagnostic and reporting tools for brucellosis.
- The Rose Bengal Test (RBT) has an accuracy level of 95-98% and is inexpensive when considered as a test for brucellosis diagnosis in health facilities.
- The locally available rapid, commercial, serological tests showed poor performance and higher cost than the RBT as frontline tests
- Training on the use of RBT for brucellosis in health facilities can improve routine diagnosis of brucellosis.
- A large scale, robust evaluation of the RBT for brucellosis in suspected patients could provide critical information on the feasibility of a national control program and how to scale it up countrywide.

BACKGROUND AND PROBLEM STATEMENT

The Tanzania One Health policy for the control and elimination of zoonoses list brucellosis as the sixth priority disease [1]. Brucellosis is among the most common bacterial zoonoses globally affecting wild animals, livestock and humans caused by bacteria of the genus ***Brucella***. The bacteria can spread from animals to humans. People can contract the disease by consuming unpasteurized dairy products from animals that are infected with the bacteria such as cows and goats. Previous studies have shown that up to 500,000 new cases occur globally each year and in northern Tanzania, up to 7% of patients presenting to health facilities suffer from illness due to brucellosis [1,2]. It affects mostly pastoral communities and is widespread in many low-income countries [1,2].

Brucellosis is poorly diagnosed due lack of appropriate diagnostics and limited awareness among medical practitioners [1], however its symptoms may include joint and muscle pain, fever, weight loss and fatigue. Some people rarely develop stomach pain and cough. Brucellosis is difficult to treat, but is managed with antibiotics. The recommended antibiotic is doxycycline in combination with rifampicin or gentamycin for 6-8 weeks [3], in Tanzania doxycycline in combination with gentamycin are commonly used while rifampicin is reserved for Tuberculosis treatment. The national surveillance guidelines for brucellosis in Tanzania recommend that all patients presenting to health facilities with brucellosis-consistent symptoms should be tested with RBT, followed by a confirmatory serological test [2,3]

In most primary healthcare facilities a range of locally available, commercial tests, with reported poor performance, are used [5,8]. Slow and tedious, paper-based reporting systems often cause delays in reporting of the disease [6,7]. Low community awareness about brucellosis has also led to increased transmission and impacts of brucellosis [4,6]. The cost of brucellosis misdiagnosis is an important element of the total public and private impacts of the disease. Misdiagnosed brucellosis is costly, including reduced income due to prolonged illnesses, prescriptions of inappropriate drugs, and repeat visits to health facilities, many of which end in misdiagnosis of illness due to inaccurate tests [5,8].

The running costs of currently available test options in northern Tanzania have not been previously evaluated [5]. Although some studies have previously assessed diagnosis of human brucellosis in Tanzania, very few studies have focused on the reporting of the disease [6,7]. A comprehensive evaluation of test performance and costs of the current test options would advise the improvement of brucellosis diagnosis in the East and Central Africa region. This stage is essential to mitigate some of the impacts of the disease. The assessment of frontline health workers awareness of brucellosis and provision of electronic-based technology for reporting brucellosis cases could improve of brucellosis diagnosis and reporting in Tanzania and mitigate some of the impacts of the brucellosis.

POLICY OPTIONS

- Knowledge on brucellosis diagnosis among healthcare workers is crucial to the mitigation of disease impacts. Awareness sessions and trainings were conducted among frontline health workers (Figure 1). After training, trainees were able to use RBT for diagnosis of brucellosis. RBT is simple and easy to use by health workers with limited laboratory

technology. Therefore, a coordinated approach to brucellosis testing in health facilities is feasible as part of a disease control strategy.

- The RBT is an inexpensive and accurate and therefor appropriate for system-wide use in health facilities. As presented in Figure 2, an RBT test (0.7 USD) costs almost USD 2.00 less than a cELISA test (2.5 USD), half the cost of the commercial rapid tests (1.1 USD) and, with an accuracy of 95-98%; it outperforms other tests on the market.
- Consideration of faster, electronic-based reporting systems to improve surveillance of brucellosis e.g. AfyaData mobile reporting tool [6].

Fig 1: Training of frontline healthcare workers on the use of the RBT for brucellosis testing in Kilosa district, Morogoro. (Photo courtesy of Mligo Belinda)

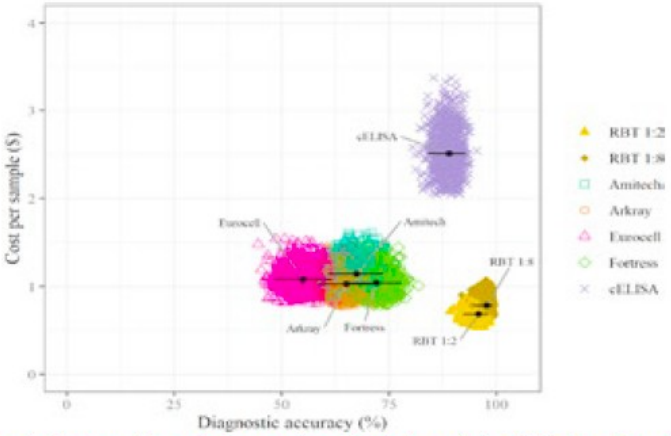


Fig 2: Cost per sample of each of tests vs diagnostic accuracy in testing for brucellosis. *RBT - Rose Bengal test, cELISA - competitive enzyme-linked immunosorbent assay [image credits- Lukambagire *et al.*, Sci Rep11, 5480 (2021)].

CONCLUSIONS AND RECOMMENDATIONS

The RBT is accurate, inexpensive, simple to use, and can be implemented as a frontline test in health facilities in diagnosis of brucellosis. Other control activities include training seminars for frontline health workers to raise their awareness on brucellosis, and improved laboratory diagnosis and surveillance of brucellosis cases in people and animals to enhance Tanzania's capacity to manage and control the disease. Ultimately, a system-wide, coordinated application of the RBT and an appropriate confirmatory test is needed that would significantly improve current data on human brucellosis, facilitating ongoing control strategies.

NOTE: The cover photographs show; 1. A local livestock and produce market in Ngorongoro district. Livestock keeping is an important economic activity for many Tanzanians, with cattle, sheep and goats playing a central role in brucellosis transmission to people. 2. Serology test kit for brucellosis used in a health facility in Arusha region, northern Tanzania. Previous studies have shown many of test kits currently used are less accurate and cost more than the recommended RBT (Photos courtesy of L.A.S and Jo Halliday).

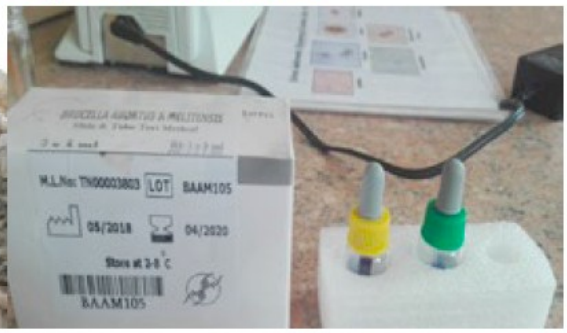
ACKNOWLEDGMENTS

This research was supported by the DELTAS Africa Initiative Afrique One-ASPIRE scholarship scheme (Afrique One-ASPIRE/DEL-15-008), the Foundation Pierre Fabre, and the Zoonoses and Emerging Livestock Systems Program (grant numbers BB/L018845, BB/N503563/1 and BB/L017679, respectively). Ethical clearance in Tanzania was approved by KCMUCo Ethics Committee (698) and the National Institute of Medical Research, Tanzania (NIMR/HQ/R.8c/Vol. I/1140) and (NIMR/HQ/R.8a/vol. IX/3235).

AUTHORS DETAILS

[1]Sokoine University of Agriculture; [2]Kilimanjaro Clinical Research Institute; [3]Ministry of Health, Community Development, Gender, Elderly and Children; [4]The Nelson Mandela African Institution of Science and Technology, [5]Kilimanjaro Christian Medical University College.

Uchunguzi wa Ugonjwa wa Brusela: Sifa Za Utendaji, Utoaji Taarifa na Gharama ya Vipimo Maalum



Lukambagire AS[1,2], Mligo JB1, Mwakapeje E[3], Mathew C[1], Shirima G4, Karimuribo ED[1], Kazwala R[1], Mmbaga BT[2,5]

UJUMBE MAALUM

- Vituo vingi vya huduma za afya vijijini havina vitendea kazi kwa ajili ya uchunguzi na utoaji taarifa za ugonjwa wa brusella.
- Kipimo cha Rose Bengal Test (RBT) kina kiwango cha usahihi wa ugonjwa wa brusela wa kiasi cha asilimia 95 hadi 98 na ni cha gharama nafuu hivyo kinafaa kuzingatiwa kama kipimo cha uchunguzi wa ugonjwa wa brusela katika vituo vya kutolea huduma ya afya.
- Vipimo ya uchunguzi wa haraka vilivyopo na kutumika nchini vimethibitika kuwa duni katika uchunguzi wa utambuzi wa ugonjwa wa brusela na gharama yake ni kubwa kuliko kipimo maalum cha RBT kwa minajili ya kutumika kama vipimo vya uchunguzi katika vituo vya kutolea huduma ya afya.
- Mafunzo juu ya matumizi ya kipimo maalum cha RBT juu ya brusela katika vituo vya kutolea huduma ya afya yanaweza kuboresha utambuzi wa ugonjwa wa brusela.
- Tathimini ya kiwango kikubwa na thabiti juu ya kipimo maalum cha RBT kwa wagonjwa wanaoshukiwa kuwa na ugonjwa wa brusela inaweza kutoa taarifa muhimu juu ya taswira ya mpango wa kitaifa wa kudhibiti ugonjwa wa brusela na jinsi ya kuupanua nchi nzima.

UTANGULIZI NA CHIMBUKO LA TATIZO

Sera ya Afya Moja ya Tanzania ya kudhibiti na kutokomeza magonjwa yanayoenezwa na wanyama imeorodhesha ugonjwa kati ya magonjwa sita yanayopewa kipaumbele[1]. Brusela ni moja ya magonjwa yanayosabishwa na vimelea aina ya bakteria unaoenezewa kwa kasi mno duniani ambapo unaathiri wanyama pori, mifugo na wanadamu. Ugonjwa huu uambukizwa na bakteria wa jamii ya Brucella. Bakteria inaweza kuenea kutoka kwa wanyama kwenda kwa wanadamu. Watu wanaweza kuambukizwa ugonjwa kwa kutumia chakula kilichotengenezwa na maziwa ambayo hayajachemushwa kutoka kwa wanyama ambao wameambukizwa na bakteria kama ng'ombe na mbuzi. Taarifa za tafiti za hapo awali zimeonyesha kuwa hadi visa vipya 500,000 vinatokea ulimwenguni kila mwaka, na upande wa kaskazini mwa Tanzania, hadi asilimia 7 ya wagonjwa wanaohudhuria vituo vya vya kutolea huduma ya afya wanaugua na brusela [1,2]. Ugonjwa huu huathiri zaidi jamii za wafugaji na umeenea zaidi katika nchi za kipato cha chini [1,2].

Ugonjwa wa brusela haugunduliki kirahisi kwa sababu ya ukosefu wa vifaa sahihi vya uchunguzi na uelewa mdogo kati ya watoa huduma ya afya[1], hata hivyo dalili zake zinaweza kujumuisha maumivu ya viungo na misuli, homa, kupungua uzito na uchovu. Watu wengine hupata maumivu ya tumbo na kikohozi kwa nadra. Ugonjwa wa brusela ni vingumu kutibika, lakini unatibika na dawa aina ya antibiotikisi. Dawa ya antibiotikisi inayopendekezwa ni aina "doxycycline" ikitumika pamoja na "rifampicin" au "gentamycin" kwa wiki 6-8 [3]. Hata hivyo kwa Tanzania "doxycycline" inatumika pamoja na "gentamycin" wakati "rifampicin" ikitengwa mahususi kwa ajili ya matibabu ya kifua kikuu. Mwongozo wa kitaifa ya ufuatiliaji wa ugonjwa wa brusela nchini Tanzania unapendekeza kwamba wagonjwa wote wanaohudhuria vituo vya kutolea huduma ya afya na dalili zinazofanana na za brusela wanapaswa kupimwa na kipimo maalum cha RBT, ikifuatiwa na kipimo kingine kuthibitisha matokeo ya awali [2,3]. Vituo vya kutolea huduma ya afya vingi vya vijijini havina uwezo wa kutibu vyema wagonjwa wa brusela [5,8].

Katika vituo vingi vya huduma ya afya ya msingi, vipimo vinavyopatikana katika soko la kibiashara vinatumika kupimia wagonjwa wa brusela ambavyo utendaji wake unaripotiwa kuwa uko chini [5,8]. Mifumo ya kutumia karatasi kutolea taarifa ni ya polepole sana, hivyo inasababisha ucheleweshaji wa upatikanaji wa taarifa za ugonjwa [6,7]. Uelewa mdogo wa jamii kuhusu ugonjwa wa brusela pia umesababisha kuongezeka kwa maambukizi na athari za brusela [4,6]. Madhara ya utambuzi usiosahihi wa ugonjwa wa brusela ni jambo muhimu na kutilia maanani kutoka na athari za ugonjwa huo kwa jamii. Ugonjwa wa brusela ukikosewa kugunduliwa sahihi unasababisha gharama kubwa. Gharama hizi ikiwa ni pamoja na madhara ya kupunguka kwa kipato kutokana na kuugua kwa muda mrefu, kusababisha kutumia dawa zisizosahihi

hivyo kutotibika. Aidha unasababisha mgonjwa kurudi mara kwa mara kwenye vituo vya kutolea huduma ya afya kufuatilia matibabu, ambapo hata hivyo huishia kupata matokeo ya ugonjwa yasiyosahihi kwa sababu ya kutumika vipimo visivyo na ubora sahihi [5,8].

Gharama za uendeshaji wa vipimo vinavyotumika kwa sasa katika eneo la kaskazini mwa Tanzania hajawahi kutathminiwa [5]. Ijapokuwa kumekuepo na tafiti zingine hapo awali zilizotathmini utambuzi wa ugonjwa wa brusella kwa binadamu nchini Tanzania, ni tafiti chache sana ambazo zililenga kuripoti ugonjwa huo [6,7]. Tathmini kamili ya utendaji wa vipimo na gharama kwa vipimo vya uchuguzi vinavyotumika sasa zitaweza kutoa mwelekeo unaolenga kuboresha utambuzi wa ugonjwa wa brusela katika ukanda wa Africa ya Mashariki na Kati. Hatua hii ni muhimu kupunguza athari zingine zinazotokana na ugonjwa. Tathmini ya uhamasishaji wa wafanyikazi wa afya walio mstari wa mbele juu ya ugonjwa wa brusela na utoaji wa taarifa za wagonjwa wa brusela kwa teknolojia ya elektroniki, itawezesha kuboresha utambuzi na kuripoti ugonjwa huu nchini Tanzania. Hatua hii itawezesha kupunguza athari zitokanazo na ugonjwa wa brusela.

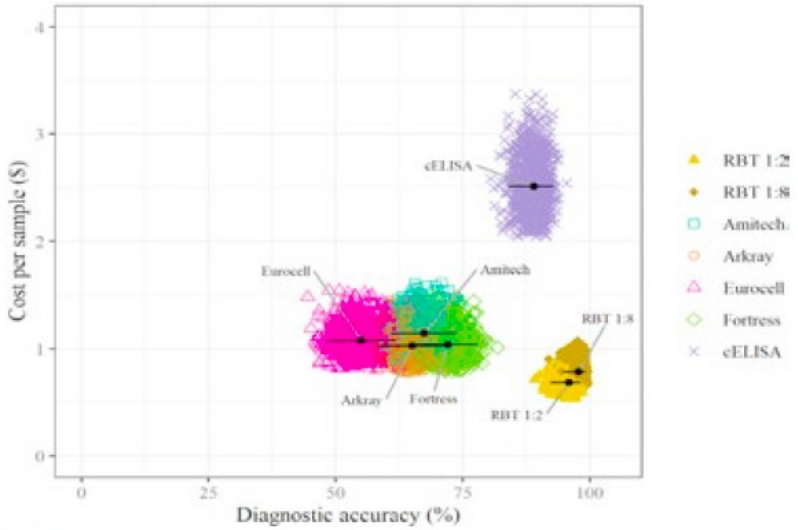
MAONI KISERA

Maarifa juu ya utambuzi wa ugonjwa wa brusela kati ya wafanyikazi wa afya ni muhimu kwa kupunguza athari za ugonjwa. Mafunzo na uhamasishaji yalifanyika kwa wafanyikazi wa afya wa vituo vya kutolea huduma ya afya kama inavyooneka kwenye Kielelezo namba 1. Baada ya mafunzo, washiriki waliweza kutumia kipimo maalum cha RBT kugundua ugonjwa wa brusela. Kipimo maalun cha RBT ni cha bei nafuu na rahisi kutumiwa na wafanyikazi wa afya walio na ujuzi mdogo wa teknologia ya maabara. Kwa hivyo, njia iliyoratibiwa ya upimaji wa ugonjwa wa brusela katika vituo vya kutolea huduma ya afya inawezekana kuwa sehemu ya mpango mkakati wa kudhibiti magonjwa.

- Kipimo maalum cha RBT ni cha gharama nafuu na kinatoa majibu sahihi, kwa hivyo kinafaa kutumika katika mfumo mzima ya vituo vya kutolea huduma ya afya nchini. Kama ilivyoainishwa kwenye Kielelezo namba 2, uchunguzi kwa kutumia kipimo maalum cha RBT (0.7 USD) unagharimu karibu Dolla za Marekani (USD) 2.00 chini pungufu kulingana na kipimo cha cELISA (2.5 USD), karibia nusu ya gharama ya vipimo vingine vinavyouzwa (1.1 USD), na kwamba kina usahihi wa kutambua vimelea vya ugonjwa wa brusela kwa kiasi cha asilimia 95-98. Aidha, kipimo maalum cha RBT kinafanya uchunguzi kwa usahihi kuliko vipimo vingine vinavyouzwa kwa ajili ya uchunguzi wa ugonjwa wa brusela.
- Kuna haja ya kufikiria matumizi ya mifumo ya haraka, ya kielektroniki ya kutoa taarifa ili kuboresha ufuatiliaji wa ugonjwa wa brusela, kwa mfano mfumo wa utoaji taarifa kwa njia ya simu wa AfyaData [6]

Kielelezo 1: Mafunzo ya wafanyikazi wa huduma ya afya juu ya matumizi ya kipimo maalum cha RBT kwa upimaji wa ugonjwa wa brusela wilaya ya Kilosa, Morogoro.

(Picha kwa hisani ya Mligo Belinda)



Kielelezo 2: Gharama ya uchunguzi kwa kila sampuli dhidi ya usahihi wa utambuzi katika upimaji ugonjwa wa brusela. * Kipimo maalum cha RBT - Rose Bengal Test, cELISA (Lukambagire et al., Sci Rep 11, 5480 (2021).

HITIMISHO NA MAPENDEKEZO

Kipimo maalum cha RBT kinapima kwa usahihi, gharama yake ni nafuu, ni rahisi kutumia, na kinaweza kutumika kama kipimo cha kugundua ugonjwa wa brusela katika vituo vya kutolea huduma ya afya. Shughuli zingine za kudhibiti ni pamoja na kuendesha semina za mafunzo kwa wafanyikazi wa afya ili kuongeza uelewa wao juu ya ugonjwa wa brusela. Mafunzo haya pia yataboresha uwezo wa utambuzi wa kimaabara na ufuatiliaji wa matukio ya ugonjwa wa brusela kwa watu na wanyama ili kuongeza uwezo wa Tanzania wa kutibu na kudhibiti ugonjwa wa brusela. Hatimaye, matumizi ya mfumo mzima, matumizi yaliyoratibiwa ya kipimo maalum cha RBT na matumizi sahihi ya kipimo cha kuthibitisha uchunguzi kinahitajika. Hii itawezesha kuboresha takwimu za sasa juu ya ugonjwa wa brusela kwa binadamu na kuwezesha mikakati ya udhibiti inayoendelea.

KUMBUKA: Picha za ukurasa wa mbele zinaonyesha; 1. Soko la mifugo na mazao yake katika wilaya ya Ngorongoro. Ufugaji ni shughuli muhimu ya kiuchumi kwa watanzania wengi, ikiwa ni pamoja na ng'ombe, kondoo na mbuzi ambao wanachangia kwa kiasi kubwa katika usambazaji wa ugonjwa wa brusela kwa watu. Picha ya 2. Vipimo vya kupima ugonjwa wa brusela vinayotumika katika kituo cha kutolea huduma ya afya katika mkoa wa Arusha, kaskazini mwa Tanzania. Tafiti za hapo awali zinaonyesha vipimo vingi kwa uchunguzi vinavyotumiwa hivi sasa havina usahihi na hugharimu zaidi ya kipimo maalum cha RBT kinachopendekezwa (Picha kwa hisani ya L.A.S na Jo Halliday).

SHUKURANI

Utafiti huu uliwezeshwa na mpango wa udhamini wa masomo wa Afrique One-ASPIRE kwa kupitia mpango wa DELTAS Afrika (Afrique One-ASPIRE/DEL-15-008) pamoja na mradi wa ZELS unaotafiti uibukaji wa maradhi katika mifumo ya ufugaji (BB/L018845, BB/N503563/1 and BB/L017679). Utafiti huu ulipewa ruhusa kwa kupitia kamati ya maadili za utafiti ya KCMUCo (cheti na.698) pamoja na Taasisi ya Taifa ya Utafiti wa Magonjwa ya Binadamu (NIMR/HQ/R.8c/Vol. I/1140). Na(NIMR/HQ/R.8a/vol. IX/3235).

REFERENCES

1. Dean, et al. Global Burden of Human Brucellosis: A Systematic Review of Disease Frequency. PLoS Negl Trop Dis. 2012;6. doi:10.1371/journal.pntd.0001865
2. United Republic of Tanzania (URT). The United Republic of Tanzania One Health Strategic Plan 2015-2020.
3. Corbel, M. J., Food and Agriculture Organization of the United Nations, World Health Organization & World Organization for Animal Health. Brucellosis in Humans and Animals; World Health Organization (WHO Press, 2006).
4. United Republic of Tanzania (URT). Guidelines for Surveillance of Prioritized Zoonotic Diseases for Human and Animal Health in the United Republic of Tanzania. Gov Rep. Dar es Salaam; 2018. doi:10.1017/CBO9781107415324.004.
5. Lukambagire, et al. Performance characteristics and costs of serological tests for brucellosis in a pastoralist community of northern Tanzania. Sci Rep 11, 5480 (2021). https://doi.org/10.1038/s41598-021-82906-w
6. Mligo, et al. Frontline health workers' awareness and use of E-based technology to enhance reporting of human brucellosis in rural setting in Tanzania. MSc Thesis; Sokoine University of Agriculture
7. Nonga HE & Mwakapeje E. Trends of human brucellosis in pastoralist communities based on hospital records during 2013–2016 in Ngorongoro District, Tanzania. Tanzania Veterinary Journal; Vol. 32 No. 1 (2017).
8. de Glanville, et al. Poor performance of the rapid test for human brucellosis in health facilities in Kenya. PLoS Negl Trop Dis. 2017;11: 1–15. doi:10.1371/journal.pntd.0005508.

Appendix 2: Ethical Clearance for Human Participant Research

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
Email: nimrethics@gmail.com

Permanent Secretary (Health)
Ministry of Health, Community Development, Gender, Elderly & Children
Government City Mtumba, Health Road
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8c/Vol. I /1627

20th November, 2020

Prof. Venance Maro
Kilimanjaro Christian Medical Centre
P O Box 3010
Moshi

RE: APPROVAL FOR EXTENSION OF ETHICAL CLEARANCE

This letter is to confirm that your application for extension on the already approved proposal: Molecular epidemiology of brucellosis in Northern Tanzania (Maro V. et al), has been approved.

The extension approval is based on the progress report dated 21st October, 2020 on the project, Ref. NIMR/HQ/R.8a/Vol. IX/2079, dated 03rd December, 2015. Extension approval is valid until 02nd December, 2021.

The Principal Investigator must ensure that other conditions of approval remain as per ethical clearance letter. The PI should ensure that progress and final reports are submitted in a timely manner.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Abel Nkono Makubi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

Appendix 3: Ethical Clearance for Animal Research

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: nimrethics@gmail.com

Ministry of Health, Community Development, Gender, Elderly & Children
University of Dodoma, College of Business Studies and Law
Building No. 11
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8a/Vol. IX/3102

04rd June, 2019

Coletha Mathew
Sokoine University of Agriculture
C/o Prof. Kazwala Rudovick
Sokoine University of Agriculture
P.O. Box 3021
Morogoro

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Approaches towards brucellosis control and prevention in Tanzania (Mathew C. et al), whose supervisor is Prof. Kazwala Rudovick of Sokoine University of Agriculture has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Morogoro, Mara, Kagera, Arusha and Kilimanjaro regions.

Approval is valid for one year: 04th June 2019 to 03rd June 2020.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

CC: Director, Health Services -TAMISEMI, Dodoma
RMO of Morogoro, Mara, Kagera, Arusha and Kilimanajaro regions
DMO/DED of respective districts

Appendix 4: Study Questionnaire for Health Facility Diagnostic Survey

HEALTH FACILITY DIAGNOSTICS AND REPORTING SURVEY QUESTIONNAIRE
To be filled by field staff only

Human brucellosis is a considerable health problem in Tanzania. We are interested to obtain an accurate picture of the current situation of brucellosis testing and results to identify key areas for future research and capacity building.
Key aims of this study are to:

1- Collect records data on current brucellosis testing practices
2- Evaluate the current brucellosis occurrence, reporting and practices

We are conducting a survey to obtain a picture of the current testing capacity and clinical impact of brucellosis in the populations attending hospitals and health centres across Arusha region. Also the study intends to find out details of the tests that are used to define brucellosis cases and the treatment regimens recommended. The cost of brucellosis screening will also be evaluated. This will provide an overall picture of the impact of brucellosis in the region.

We are requesting your help to extract and report data from the Lab at your hospital. We can use the lab record books in use at the health facility that you work at, to gather the information summarised below.

If you have any questions or encounter a problem with the form please feel free to contact study personnel at provided numbers

PRELIMINARY INQUIRIES FROM HEALTH ADMINISTRATIVE/ MANAGEMENT STAFF:

A. <u>At the RMO's Office:</u>

The team will attempt to acquire the following information;
Date: … Name & contact of interviewee: … … Initials of interviewer:

1. Brief introduction of the research team, aims and objectives
2. Presentation of ethical clearance documents and data collection tools to be used for the exercise.
3. Request official documentation (letter) authorizing the conduct of this study in the region, copied to the respective District Medical Officers (DMOs)
4. Request names and contact details for the members of the regional Health Management Team responsible for:
    a. supervision and oversight of health laboratories (Regional Lab Technologist),
    b. records and reports for the region
    c. procurement of regional level reagents and consumables.

Date	Name	Contact	Designation/ Office	Location	Comments

5. Discuss inclusion of a representative (preferably from the RLT department) to accompany the research team during the survey exercise.
6. Brief overview of the structure and layout of designated district hospitals/ health facilities as well as their classification

District Name	Designated Hospital/HC	Classification	Name of Contact	Designation/ Office	Comments

NOTE: In the absence of the RMO, the team should attempt to contact the Acting/Deputy RMO and share this key information with a commitment to follow up the visit with another attempted appointment as well as a written report of the issues discussed.

The team will then attempt to contact the identified RLT, Records Manager as well as procurement manager at the RMOs office.

B. **At the Regional records department**:

The team will attempt to acquire the following information;

Date: … Name & contact of interviewee: … … Initials of interviewer:

a. Can you provide a list of all the registered health facilities in the region?

District	Health facility	Diagnostic lab category	Contact Person & No.	Brucellosis reported Y/N	Comments

b. Can you explain the process of records and reporting from the lowest level health facility to the pooled regional records; record book used and source of data recorded therein (details included in flow diagram).

i. Do you have a list of all the registered district health facilities in the region Y N

ii. Can you give us the details of the person responsible for collection of records at each district health facility: Name:… Contact: … Alternative: … (table)

iii. Do you have pooled/ summary records from all the above mentioned health facilities Y N

iv. Do you have records of all health facilities reporting cases of brucellosis from 2012 to date? Y N

C. <u>At the Regional Procurement Office/ Contact Person</u>

Date: … Name & contact of interviewee: … … Initials of interviewer:

a. What laboratory reagent procurement system was implemented in 2012?
    i. Was the central government procurement process implemented in your regional office in 2012?
    b. What is the MSD procurement system?
    c. What is the alternative system if MSD doesn't have it?
b. Can you give an overview of the current laboratory reagents procurement process at the regional level? (summarise in a schematic flow diagram)
c. For how long has this system (b) been implemented?
d. What reagents/ kits are currently approved brucellosis testing in humans in this region?
e. What reagents/ kits are currently in use for brucellosis testing in humans in this region (district)?

S/N	Test Name	Manufacturer	Tz Supplier	Comments
				e.g. approval status

f. Who approves tests for brucellosis at the regional level …; national level …?
g. Where are these reagents sourced from?
h. Contact persons at each district level health facility who process procurement requests for laboratory reagents (including brucellosis).

D. <u>At the Regional Laboratory Technologist's Office:</u>

Date: … Name & contact of interviewee: … … Initials of interviewer:

Introductions and an overview of the study objectives, planned exercise and focus/ purposed aims of the survey and information collected.

i. Can you provide a list of registered district health facilities in the region reporting results on brucellosis testing? Y N (if yes, include details in table)

District	Health facility	Diagnostic lab category	Contact Person & No.	Brucellosis reported Y/N	Comments

ii. What are the conditions for a facility to be registered to conduct brucellosis testing?
    a. List if any …
    b.

iii. Identify contact persons at District health facilities who can be contacted to facilitate the survey on the ground (lab managers, district lab technologists as well as known/authorised suppliers of laboratory reagents and services).

NOTE: As with the RMO, in absence of the individual identified at each of these key departments, their proxy or acting counterpart should be contacted and an effort made to re-visit and brief the contact persons at a later appointment. A written report of issues discussed during the visit should also be sent to the relevant contact person to officially inform them of the visit and planned activities.

The team will then proceed to the designated district hospital for each district and there, following a similar approach try to contact and inform the DMO, DLT and Records and procurement contact persons.
Attempt to collect similar information at the district level as was inquired for at the regional level;
Request lists of registered health and testing facilities operating within the district as well as contact information for the notifiable persons to facilitate the exercise. One member of the research team will the attempt to contact individuals identified at the different facilities by phone and where successful;

a. Give a brief introduction and summary of the study scope and objectives
b. Identify any procedures or authorisations required to visit the health/ testing facility
c. Fix an appointed date (and appropriate time frame) to visit the health facility
d. Request that contact persons and MTUHA Master and Lab log books be made available for the planned visit so as to expedite the process and have minimal intrusion or interference in the day-to-day operations of the facility

District Name	Health facility	Classification	Name of Contact	Designation/ Office	Comments

Where contact with identified parties is not successful (or where no contact information has been provided) I will make three more attempts to contact the persons identified before notifying the district office to request an alternative contact at the facility in question.

ONLY for facilities identified at the RLT and DLT offices as conducting brucellosis testing will the team venture a physical visit.
All other institutions where no contact was made and are not identified as facilities reporting brucellosis cases or conducting testing will simply be noted as non-participating facilities in the study

BRUCELLOSIS TEST & CASE RECORD SHEET.

NAME OF THE HEALTH FACILITY/HOSPITAL: ……
Date: … Name & contact of interviewee: … … Initials of interviewer:

Details of all individuals and their role who have contributed information to complete this form.

Name	*Role*	*Contact Number*

DATA TABLES

In each table below, please record the following details for each month & year:

Number tested for Brucellosis – this is the total number of patients who had a brucellosis test performed (this can be any kind of brucellosis test) in this month – including adults, infants, inpatients and outpatients combined.

Number tested for positive Brucellosis – this is the total number of patients who had a brucellosis test performed in this month and were positive based on the test in use at your hospital

Total number OPD Patients – this is the total number of patients (including adults and infants) who attended an outpatient department at your hospital in this month

Total number In Patients – this is the total number of patients (including adults and infants) who were admitted to any ward or department at your hospital in this month

***YEAR 2012* Records**

Month	Number tested for Brucellosis	Number positive for Brucellosis	Total number of OPD patients	Total number of IPD patients
January				
February				
March				
April				
May				
June				
July				
August				
September				
October				
November				
December				
TOTAL				

Lab records 2012

Month	***Number tested for Brucellosis***	***Number positive for Brucellosis***
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		
TOTAL		

YEAR 2013 Records

Month	Number tested for Brucellosis	Number positive for Brucellosis	Total number of OPD patients	Total number of IPD patients
January				
February				
March				
April				
May				
June				
July				
August				

September				
October				
November				
December				
TOTAL				

Lab Records 2013

Month	***Number tested for Brucellosis***	***Number positive for Brucellosis***
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		
TOTAL		

YEAR 2014

Month	**Number tested for Brucellosis**	**Number positive for Brucellosis**	**Total number of OPD patients**	**Total number of IPD patients**
January				
February				
March				
April				
May				
June				
July				
August				
September				
October				
November				
December				
TOTAL				

Lab records 2014

Month	***Number tested for Brucellosis***	***Number positive for Brucellosis***
January		
February		

March		
April		
May		
June		
July		
August		
September		
October		
November		
December		
TOTAL		

YEAR 2015

Month	**Number tested for Brucellosis**	**Number positive for Brucellosis**	**Total number of OPD patients**	**Total number of IPD patients**
January				
February				
March				
April				
May				
June				
July				
August				
September				
October				
November				
December				
TOTAL				

Lab records 2015

Month	***Number tested for Brucellosis***	***Number positive for Brucellosis***
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		
TOTAL		

YEAR 2016

Month	Number tested for Brucellosis	Number positive for Brucellosis	Total number of OPD patients	Total number of IPD patients
January				
February				
March				
April				
May				
June				
July				
August				
September				
October				
November				
December				
TOTAL				

Lab records 2016

Month	***Number tested for Brucellosis***	***Number positive for Brucellosis***
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		
TOTAL		

YEAR 2017

Month	Number tested for Brucellosis	Number positive for Brucellosis	Total number of OPD patients	Total number of IPD patients
January				
February				
March				
April				
May				
June				
July				
August				
September				

October				
November				
December				
TOTAL				

Lab records 2017

Month	***Number tested for Brucellosis***	***Number positive for Brucellosis***
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		
TOTAL		

YEAR 2018

Month	**Number tested for Brucellosis**	**Number positive for Brucellosis**	**Total number of OPD patients**	**Total number of IPD patients**
January				
February				
March				
April				
May				
June				
July				
August				
September				
October				
November				
December				
TOTAL				

Ab records 2018

Month	***Number tested for Brucellosis***	***Number positive for Brucellosis***
January		
February		

March		
April		
May		
June		
July		
August		
September		
October		
November		
December		
TOTAL		

DIAGNOSTIC TEST INFORMATION

Date: … Name & contact of interviewee: … … Initials of interviewer:

Please give the name (or names) of the test(s) used for brucellosis testing at the health facility

Please include:

- Are there Brucella test reagents in the lab currently? Y N
- the name of the manufacture of the test reagents (File name)
- the exact name of the test kit & any product number available
- Details about the time period over which different tests have been used e.g. if different tests have been used at different times in the period Jan 2012 to May 2018.

Please describe the procedure that you use to perform the brucellosis test that you use most frequently at the health facility where you work.

- Please include details on the following features of the test as appropriate. For sections that are not appropriate or not done please record 'NA' or 'Not Done':
-

1 – Details of the type and volume of test sample used – e.g. serum, whole blood, plasma, other

-
-

2 – details of any dilution steps for the test sample e.g. do you test at a single dilution or use serial dilutions of sample?

-
-
-
-

3. Details of any control samples used and how frequently controls are tested e.g. do you use a positive control, do you use a negative control, how often are these used?

-
-
-
-
-

4 - Details of the test procedure – e.g. how the sample is mixed with test reagents including details of the volumes of reagents used

-
-
-
-
-

5. Details of the test process – e.g. how long the test takes and if any mixing, incubation steps are needed

-
-
-
-

6. Details of the interpretation of test results – e.g. how is a sample defined as positive, how is a sample defined as negative, how is a sample defined as indeterminate?
7. Details of any additional testing performed for positive, negative or indeterminate

8 – are there any circumstances where you re-test samples? Describe if any

TEST COST INFORMATION

Date: … Name & contact of interviewee: … … Initials of interviewer:

Where do you source your reagents for brucellosis testing. Please give details of the name and location, and contact information of the company/companies or pharmacy/pharmacies that you use to supply brucellosis tests.

What is the test cost to the patient (Tshs)
What is the overall cost of getting a brucellosis diagnosis to the patient (include opening file, consultation fee, common other accompanying tests, any repeat visits …
Test turn around time)

NOTES ON FORM COMPLETION Please record any comments or queries that you have about completion of this form or this data collection process?
……

We thank you for your commitment and time devoted on this important information

Appendix 5: Proof of Submission of Paper Two to Microorganisms Journal

Gmail — Abdul-Hamid Lukambagire <lukhamid@gmail.com>

[Microorganisms] Manuscript ID: microorganisms-1756690 - Submission Received

1 message

Editorial Office <microorganisms@mdpi.com> 19 May 2022 at 22:57
Reply-To: microorganisms@mdpi.com
To: AbdulHamid Settenda Lukambagire <lukhamid@gmail.com>
Cc: James M Akoko <jamesakoko@yahoo.com>, Coletha Mathew <colethamat@sua.ac.tz>, Rosamystica M Sambu <mkula1969@yahoo.com>, Raphael R Mwampashi <rmwampashi@gmail.com>, Richard B Yapi <ryrichardyx@gmail.com>, Nelso B Amani <nelisob3@yahoo.com>, Judith S Njau <j.njau@kcri.ac.tz>, Bassirou Bonfoh <bassirou.bonfoh@csrs.ci>, Gabriel M Shirima <gabriel.shirima@nm-aist.ac.tz>, Blandina T Mmbaga <b.mmbaga@kcri.ac.tz>, Christopher Jacob Kasanga <chrisskasa@gmail.com>, Robab Katani <rxk104@psu.edu>, "Jo E.B. Halliday" <jo.halliday@glasgow.ac.uk>, Rudovick Reuben Kazwala <kazwala@gmail.com>

Dear Mr. Lukambagire,

Thank you very much for uploading the following manuscript to the MDPI submission system. One of our editors will be in touch with you soon.

Journal name: Microorganisms
Manuscript ID: microorganisms-1756690
Type of manuscript: Article
Title: Direct detection of Brucella species in Blood Clots from Livestock in Northern Tanzania
Authors: AbdulHamid Settenda Lukambagire *, James M Akoko, Coletha Mathew, Rosamystica M Sambu, Raphael R Mwampashi, Richard B Yapi, Nelso B Amani, Judith S Njau, Bassirou Bonfoh, Gabriel M Shirima, Blandina T Mmbaga, Christopher Jacob Kasanga, Robab Katani, Jo E.B. Halliday, Rudovick Reuben Kazwala
Received: 19 May 2022
E-mails: lukhamid@gmail.com, jamesakoko@yahoo.com, colethamat@sua.ac.tz, mkula1969@yahoo.com, rmwampashi@gmail.com, ryrichardyx@gmail.com, nelisob3@yahoo.com, j.njau@kcri.ac.tz, bassirou.bonfoh@csrs.ci, gabriel.shirima@nm-aist.ac.tz, b.mmbaga@kcri.ac.tz, chrisskasa@gmail.com, rxk104@psu.edu, jo.halliday@glasgow.ac.uk, kazwala@gmail.com
Submitted to section: Medical Microbiology, https://www.mdpi.com/journal/microorganisms/sections/medical_microbiology
Emerging Themes in Brucella and Brucellosis https://www.mdpi.com/journal/microorganisms/special_issues/emerging_themes_brucella_brucellosis

You can follow progress of your manuscript at the following link (login required):
https://susy.mdpi.com/user/manuscripts/review_info/5b9a6eb2c2b3fdce810391d1a96b0bb3

The following points were confirmed during submission:

1. Microorganisms is an open access journal with publishing fees of 2200 CHF for an accepted paper (see https://www.mdpi.com/about/apc/ for details). This manuscript, if accepted, will be published under an open access Creative Commons CC BY license (https://creativecommons.org/licenses/by/4.0/), and I agree to pay the Article Processing Charges as described on the journal webpage (https://www.mdpi.com/journal/microorganisms/apc). See https://www.mdpi.com/about/openaccess for more information about open access publishing.