**TRACK RECORD**

**Molecular epidemiology of brucellosis in northern Tanzania**

This project team has been brought together to forge new relationships between Tanzanian and UK research institutions, to build on and extend the reach of an existing up-and-running research platform with proven functionality, and to bring policy-makers into the research process, enabling influence over the research agenda, its outputs and relevance. This team will build capacity to use novel diagnostic and analytical approaches to target specific data gaps that currently inhibit the effective control of brucellosis in Tanzania.

**Prof. Dan Haydon (University of Glasgow)** provides overall coordination for the project. Haydon is Director of the Institute for Biodiversity, Animal Health and Comparative Medicine, a research group with extensive experience of both leading and collaborating with overseas research projects, particularly in East Africa. He has experience as PI and Co-PI of managing research projects on infectious disease ecology in Africa funded by MRC, BBSRC, and EU, and as co-lead (with Sarah Cleaveland) as a global partner to the Wellcome Trust funded African Institute Initiative *AfriqueOne*. Haydon has broad experience of modelling the dynamics and control of infectious disease epidemiology, and specific expertise in the design of vaccine interventions, and statistical methods for integrating molecular and epidemiological data.

**Prof. John Crump (University of Otago)** is an expert on infectious disease epidemiology in low-resource areas and worked in northern Tanzania as director of the KCMC clinical research programme for over 10 years. His recent work has demonstrated that brucellosis is a major cause of severe febrile illness among hospitalized patients in northern Tanzania and that most febrile illness is misdiagnosed as malaria. His work contributes to the improved management of severe human febrile illness in Tanzania and provides insights into disease control strategies for the economic wellbeing of poor livestock keepers.

**Prof. Paul Gwakisa (Nelson Mandela African Institute of Science and Technology)** is founding Dean of the School of Life Sciences and Bioengineering at NMAIST, a newly established academic institution in Tanzania that has a clear international and collaborative outlook. He has a proven track record establishing research laboratory capacity and educational programmes in Tanzania including an innovative postgraduate platform for Genome Sciences. He is Professor of Immunology and his research career has focused on indigenous livestock populations in Tanzania, examining genetic diversity, disease resistance and vaccine responses.

**Dr. Jo Halliday (University of Glasgow)** is an infectious disease epidemiologist with expertise in the surveillance of zoonotic pathogens in linked animal and human populations. She has worked on several field epidemiology studies in Kenya and Tanzania and is currently the research coordinator for the BBSRC-funded BACZOO project. Her recent work includes evaluation of the impact of urbanization on zoonotic disease risks in urban slum settings and surveillance policy development, with an emphasis on the potential for investments in the surveillance of endemic zoonoses to have broader scale impacts and benefits.

**Prof. Rudovick Kazwala (Sokoine University of Agriculture)** is Professor of Public Health and Veterinary Epidemiology and has made substantial contributions to the foundation of One Health research work in Tanzania. He has a long track record of successful international collaborative research and over 50 publications including several on the epidemiology of brucellosis in different animal and human populations in Tanzania. His previous work has made significant contributions to the development of disease control policy in Tanzania, including his authorship of the national rabies control strategy for Tanzania.

**Dr. Moshi Ntabaye (Kilimanjaro Christian Medical Centre)** is executive director of KCMC and leads KCMC’s Medical Education Partnership Initiative to strengthen medical education in Tanzania. This programme aims to equip a new generation of medical scientists in Tanzania with the knowledge and tools to become future leaders in academia, research and policy.

**Dr. Gabriel Shirima (Tanzania Vaccine Institute)** is principal veterinary research officer responsible for zoonoses and the production of animal vaccines at the Tanzania Vaccine Institute/Central Veterinary Laboratory. He is responsible for overall coordination and supervision of research into bacterial zoonoses, with a focus on brucellosis and tuberculosis. His academic research (dating back to his doctoral research undertaken with researchers now at the University of Glasgow) has focused on the assessment of the impacts and epidemiology of brucellosis within...
a range of livestock keeping populations in Tanzania and he has strong collaborative links with several international research and development organisations.

**Dr. Emanuel Swai (Ministry of Livestock and Fisheries Development)** is a veterinarian, who has been working with the MoLFD since early 2000 and is currently based at the Directorate of Veterinary Services. He has been very active in primary research, focusing on brucellosis and other pathogens of zoonotic and economic importance as well as in development initiatives aimed at improving livestock production and human livelihoods.

**Dr. Adrian Whatmore (Animal Health and Veterinary Laboratories Agency)** is Senior Scientist within the FAO/WHO Collaborating Centre for Brucellosis/OIE Brucellosis Reference Centre at the AHVLA. Dr Whatmore leads an active research program focussing particularly on the development and application of novel diagnostic and molecular epidemiology approaches to understanding brucellosis. He is author of more than 80 publications in peer reviewed journals and book chapters and has considerable experience building diagnostic capacity in low and middle income countries.

Previous and ongoing work conducted by members of this international research team has made significant contributions to our understanding of the impact, epidemiology and control of zoonotic diseases in Tanzania. This project builds directly on the research platform provided by the ongoing BBSRC and NIH funded study: *The impact and social ecology of bacterial zoonoses in northern Tanzania* (BACZOO, BB/J010367/1). The BACZOO study involves partners at the University of Glasgow (Haydon & Halliday); Sokoine University of Agriculture (Kazwala); Kilimanjaro Christian Medical Centre (Ntabaye) and University of Otago (Crump) who are included in this proposed collaborative team. BACZOO is currently generating data on the incidence, morbidity, and mortality attributable to bacterial zoonoses among humans with fever, and investigating the social ecology of bacterial zoonoses among livestock owning communities in northern Tanzania. *Brucella* is one of the three key pathogens included in BACZOO and members of this team have recently published an article on "Brucellosis in low-income and middle-income countries", (Halliday & Crump, 2013), emphasizing the need for a One-Health approach to the control of this disease. The network of host institutions for this study provides expertise, experience and well-established infrastructure for field epidemiological studies. **University of Glasgow (UG)** is a recognized centre of excellence for research in infectious disease ecology, molecular and quantitative epidemiology and one-health science. UG has extensive experience of providing training in these techniques and of developing and applying this expertise in collaboration with African institutions and researchers. **Animal Health and Veterinary Laboratories Agency (AHVLA):** The Brucellosis Reference Unit at Weybridge has extensive experience in advising in all matters relating to diagnosis, characterisation and control of *Brucella* in many parts of the world and has a large network of international collaborators. As a reference centre for *Brucella*, the AHVLA will play a key role in providing expertise and diagnostic support to the KCMC and TVLA laboratories in Tanzania and in building sustainable capacity in Tanzania. **Kilimanjaro Christian Medical Centre (KCMC)** is a referral hospital serving over 11 million people with extensive engagement in research projects related to malaria and non-malaria febrile illness. The biotechnology unit at KCMC has BSL2 and BSL3 laboratory facilities. **The Nelson Mandela African Institute of Science and Technology (NMAIST)** will host the Tanzanian-based PDRA on this project. NMAIST is part of a network of pan-African training institutions, with a primary focus on postgraduate and post-doc training in Science, Engineering and Technology. They have a remit to stimulate intensification of agricultural production, placing emphasis on linkages between society, local industry and local technological response to local needs. **Sokoine University of Agriculture (SUA)** is the base institution for most veterinary training and research conducted in Tanzania. **University of Otago (UG)** hosts the Centre for International Health that promotes research to improve health in under-resourced countries. The **Ministry of Livestock and Fisheries Development (MoLFD)** has the mandate for overall management and development of Tanzanian livestock and fisheries development for the sustainable achievement of the Millennium Development Goals. **The Tanzania Veterinary Laboratory Agency (TVLA)** is an executive agency of the MoLFD and includes the Tanzania Vaccine Institute (TVI). The TVLA is mandated to undertake diagnosis of animal diseases, regulate veterinary laboratories, conduct research into animal disease and produce vaccines. The organisation’s aims are to promote animal health and welfare through provision of services to livestock stakeholders in order to enhance food safety, food security and the national economy.
CASE FOR SUPPORT
Molecular epidemiology of brucellosis in northern Tanzania

Brucellosis is described by the WHO as “responsible for more sickness, misery, and economic losses than any other zoonosis”. Species-specific livestock vaccines have been used effectively as part of programs to control this disease in other parts of the world. However, in sub-Saharan Africa control programs are held back because we do not know which *Brucella* species is the primary cause of human illness, and which animal species is the most important source of human infections. This project will build on existing research platforms to generate critical species-specific data from infected animals and humans, and develop innovative latent variable models to analyse combined serological and species-specific infection data to determine the primary source of human disease and which vaccine is best targeted at which host species. This project will be conducted hand-in-hand with Tanzanian government scientists charged with formulating national policies for the control of brucellosis and will build capacity in Tanzanian laboratories to generate these critical species-specific data. The specific objectives of this project are:

**Research objective 1 - Pathogen typing:** Establishing PCR assays to detect and type *Brucella* to species level in Tanzania.

**Research objective 2 – Active human case finding:** Establishing active sample collection approaches to identify and sample high-probability human brucellosis cases to determine the *Brucella* species responsible for human illness.

**Research objective 3 – Source identification:** Quantifying the contribution of different hosts and *Brucella* species to the risk of human brucellosis using hierarchical Bayesian models.

**Impact objective – Formulating control policy:** Communicating research findings to policymakers to contribute to and facilitate the development of national brucellosis control strategy.

**Scientific significance.** Brucellosis has been identified as one of the highest priority animal diseases in sub-Saharan Africa. In Tanzania, there is evidence of widespread animal exposure and that brucellosis is an important cause of human illness. In humans, non-specific clinical signs and a lack of diagnostics for confirming acute infection contribute to marked under-reporting of brucellosis. In East Africa it is estimated that just one in every million cases of livestock brucellosis is reported to the OIE and human cases are commonly mis-diagnosed as malaria or typhoid fever. This under-reporting leads to under-estimation of the scale of the problem and lack of investment in research and control. As a consequence, major gaps in our understanding of the epidemiology and control of brucellosis remain, and a deeper understanding of the molecular epidemiology of brucellosis has been identified by DFID as a research priority.

Human brucellosis is caused by several different *Brucella* species, each of which may have a complex multi-host epidemiology. Data from Europe, North America and the Middle East indicate that *Brucella abortus* is commonly maintained in cattle and *B. melitensis* in sheep and goats. However, in sub-Saharan Africa *B. abortus, B. melitensis* (and *B. suis*) have been reported in cattle and all of these pathogens (as well as *B. ovis*) have been observed in sheep and goats. Data from sub-Saharan Africa are mostly serological and their interpretation is hamstrung by the inability to differentiate between *Brucella* species based on serology and the known capacity for transmission of several *Brucella* species between different animal host species. Consequently, we do not know whether cattle, and/or sheep and goats, are infected with *B. melitensis*, with *B. abortus* or with both or which *Brucella* species is responsible for most human illness. Current attempts to control brucellosis are hampered by the absence of information on two fundamental aspects of the epidemiology of brucellosis in sub-Saharan African. First, which pathogen species is/are the most important cause of human illness; second, which animal species constitute the reservoir and/or source populations for human infections? To address these questions, the detection and typing of *Brucella* spp. in different host species is essential.

To address both the human disease and livestock productivity impacts of brucellosis, control of infection in animals is essential and vaccination is the most successful method for achieving this. Vaccination with test and slaughter policies have contributed to the elimination of *Brucella* spp. in some developed countries but the cost and infrastructural requirements of this approach means that elimination is unlikely to be feasible in many developing country settings. However,
targeted vaccination can reduce the impacts of brucellosis and improve the livelihoods of the poor communities most affected by this disease. The most widely used vaccines are the live attenuated vaccines, *B. melitensis* Rev 1, used in sheep and goats and *B. abortus* S19, used in cattle. Assessment of a control program in small ruminants in Tajikistan showed an 80% reduction in prevalence in areas with high Rev1 vaccine uptake, and a 40% reduction in prevalence in areas with low coverage. Cost-effectiveness modeling of a vaccination campaign using Rev1 vaccine for small ruminants and S19 vaccine for cattle in Mongolia estimated that a 52% reduction in transmission between animals could be achieved and a total of 49,027 disability-adjusted life years could be averted. \textbf{Given the potential beneficial impacts of vaccination, this project is tightly focused on filling key-gaps in our understanding of the molecular epidemiology of brucellosis that would enable the use and deployment of Brucella vaccines in sub-Saharan Africa and communicating this information to appropriate policy-makers.}

**Development relevance.** Endemic zoonoses are responsible for both the vast majority of human illness (and mortality) and the greatest reduction in livestock productivity. There are strong associations between poverty, livestock keeping and zoonoses, and areas with both high livestock populations and rising demand for livestock products offer the greatest opportunity for livestock to serve as a pathway out of poverty. There are few places on the planet where this is more true than sub-Saharan Africa. This region has the world’s fastest growing human population and approximately one third of this population uses livestock as their principal currency for social and commercial transactions. Between 2003 and 2008 the Tanzanian livestock sector experienced annual growth rates of 35% for dairy cattle, 6% for beef cattle, 5% for goats and 8% for sheep and in 2007/8 the livestock sector contributed 5% of GDP. East Africa has the fastest rate of urban growth of anywhere in the world. While growing urban populations may drive demand for animal products and the expansion of urban and peri-urban livestock-keeping, connectivity between urban and rural populations is likely to be strong. The importance of different animal to human transmission routes varies across livestock production systems. In settings of nomadic or migratory animal husbandry, or on traditional smallholdings, household members are often exposed to infection by direct contact with their animals or indirectly via contaminated food. Under more settled/urban conditions, the main sources of human exposure tend to be occupational exposure (e.g. abattoir workers) and foodborne transmission. The highly dynamic nature of emerging livestock systems is likely to have significant implications for the epidemiology and relative importance of different transmission routes of Brucella. There are already indications that brucellosis risk is changing as human populations and their interactions with animals change, for example, recent studies have revealed high incidence of human brucellosis and high cattle seroprevalence in urban centres (e.g. Kampala).

The impacts of brucellosis on the livelihoods and health of poor people are considerably underdocumented but the available data are still sufficient to justify identification of brucellosis as one of the highest priority endemic zoonoses. Impacts fall disproportionately on specific groups in society. Pastoralist populations are thought to be at higher risk of brucellosis infection because of their close contact with livestock, practices of consuming raw milk products and poor access to health care services. In rural livestock-based economies women make up two-thirds of low-income livestock keepers and in many African countries women own most of the goats. Activities such as milking and processing of milk from ruminants are frequently carried out by women and children and this places them at greater risk of exposure to *Brucella*. The socioeconomic impacts of brucellosis include animal losses due to abortion, diminished milk production, cull and condemnation of infected animals, human illness causing reduced work capacity and productivity and losses of financial investments. Estimates of the economic losses of bovine brucellosis are 6-10% of income per animal. In regions where brucellosis is endemic, the social and economic consequences of reduced animal productivity combine with the multiple impacts of the chronic and debilitating multi-systemic disease caused in humans, threatening the most vulnerable socioeconomic sections of society.

At a recent international workshop on “An integrated approach to controlling brucellosis in Africa”, key priorities for work in Tanzania and the wider region were defined by Tanzanian policy makers (including co-applicants Dr. Swai & Dr. Shirima) and their regional counterparts. These included:

- Increased cooperation to encourage collaboration between medical and veterinary sectors.
Track Record & Case for Support

- Building the laboratory diagnostic capacity to type *Brucella* within Tanzania and develop collaborations between local, regional and international reference labs.
- Capacity building to train people and build the epidemiology skills needed to map *Brucella* prevalence across settings and livestock keeping systems, including data on typing of strains.
- Sharing of technical information and enhancing joint collaborative activity among key players with a one-health approach.

This project will build a multidisciplinary one-health research team of veterinary and medical scientists with a track record of working with Tanzanian policy makers and scientists to address their research priorities, build capacity in Tanzania, fill gaps in our broader understanding of *Brucella* epidemiology and develop analytical approaches that can be used to inform efficient prioritization of brucellosis control investments.

Current state of knowledge.

In Tanzania, previously collected serological data reveal considerable exposure in several livestock species and significant clinical burden in humans. Cattle surveys have demonstrated seroprevalences of 4.1% in smallholder cattle, 7.3% in more extensively managed herds, 12.2% in an agro-pastoral setting, and 15.6% in a pastoral population. In goats and sheep, seroprevalences of 4.6% and 3.4% respectively, and 6.5% in a combined small ruminant population have been recorded. In humans, seroprevalences as high as 19.5% have been observed in abattoir workers, 6.2% patients with clinical signs consistent with brucellosis at hospitals throughout northern Tanzania were seropositive and 3.5% febrile inpatients in Moshi hospitals met criteria for confirmed acute brucellosis. Previously identified risk factors for human infection in Tanzania include high-risk occupations (e.g. abattoir workers) and assisting livestock after abortion. However, there is almost no data anywhere in sub-Saharan Africa on the distribution of different *Brucella* species across host species.

Relationship to existing research.

This project provides an opportunity to add value to an ongoing BBSRC and NIH funded study: *The impact and social ecology of bacterial zoonoses in northern Tanzania* (BACZOO, BB/J010367/1). BACZOO involves collection of diagnostic samples from linked animal and human populations stratified across three different livestock systems (peri-urban, agro-pastoral and pastoral settings) in northern Tanzania, with linked questionnaire and social science surveys to examine the health, productivity and social impacts of brucellosis and other zoonoses. BACZOO is restricted to serological diagnostics but the study is collecting and archiving specimens from over 6000 animals that are available for more detailed molecular diagnostic analyses. Vaginal swabs, which are an excellent source for the recovery of *Brucella* and milk samples, in which *Brucella* are frequently shed, are routinely collected through BACZOO. BACZOO involves data collection across the spectrum of livestock systems from pastoral to peri-urban and provides a data set that can be built upon to evaluate brucellosis risks at different points along this spectrum. The project team have existing links with other research groups working on *Brucella* epidemiology in the wider region, including the collaboration between the Zoonotic Disease Unit of the Government of Kenya and CDC-Kenya who are conducting a study of animal and human *Brucella* incidence in Kajiado in southern Kenya. This project would be complementary to other projects that might be funded through the ZELS scheme, including the abortion etiology study, SEEDZ, HAZEL and AZOBAT+. The diagnostic capacities established through this project would be valuable to many of these studies and data obtained through testing of their additional samples could be integrated into our analyses.

Programme & Methodology.

To develop plans for brucellosis control it is necessary to understand which host species are infected with which *Brucella* species, and which inter-species transmission routes are most important in transmitting brucellosis to the target human population in different settings. New tools and techniques such as the molecular diagnostics and latent variable models described below are needed to achieve this, make the best possible use of serological data that are widely available but under-utilised and identify novel methods for targeting interventions for this important zoonosis.
**Research objective 1: Pathogen typing.** We will establish diagnostic test capacity to detect and type *Brucella* to species level at KCMC. The NMAIST PDRA will train in real-time and conventional PCR techniques at AHVLA. They will then work with AHVLA staff to establish these assays in Tanzania to detect *Brucella* in animal swab and milk specimens collected from cattle, sheep and goats sampled through BACZOO. Sera, vaginal swab and milk samples are being collected from cattle, sheep and goats from 45 villages in northern Tanzania through BACZOO, stratified across peri-urban, agropastoral and pastoral settings. Based on the collection rates for swab and milk samples observed to date for each sample type and setting (dependent on herd composition, animal sex and reproductive condition), the following sample numbers are expected to be available for molecular analysis in this study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Species</th>
<th>Total anticipated sample size ( ^a )</th>
<th>Estimated sample number collected from infected animals ( ^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal Swabs</td>
<td>Cattle</td>
<td>((675*3) = 2025)</td>
<td>((55*3) = 165)</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>((563*3) = 1689)</td>
<td>((46*3) = 138)</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>((563*3) = 1689)</td>
<td>((46*3) = 138)</td>
</tr>
<tr>
<td><strong>ALL</strong></td>
<td></td>
<td>(5403)</td>
<td>(441)</td>
</tr>
<tr>
<td>Milk</td>
<td>Cattle</td>
<td>((223*3) = 669)</td>
<td>((18*3) = 54)</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>((56*3) = 168)</td>
<td>((5*3) = 15)</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>((186*3) = 558)</td>
<td>((15*3) = 45)</td>
</tr>
<tr>
<td><strong>ALL</strong></td>
<td></td>
<td>(1395)</td>
<td>(114)</td>
</tr>
</tbody>
</table>

\(^a\) Based on projected swab sampling rates of 90% animals in pastoral areas and 75% in agro-pastoral and peri-urban areas. Projected milk samplings rates are 33% for cattle and goats and 10% for sheep across settings. *Estimated numbers for infected animals are based on an overall estimated seroprevalence of 8.2% in East African livestock. Positive antibody tests indicate that an animal is currently sick, chronically infected or has been infected in the last year, hence, % seroprevalence is approximately equivalent to % annual cases.*

A real-time PCR (IS711 target\(^{31,32}\)) will be established at KCMC to test a sub-set of swab and milk samples for the presence of *Brucella* spp. For each animal species, setting and sample type (e.g. cattle milk samples from pastoralist areas), a sample of at least 114 is sufficient to detect an average of 9 positive samples in each of the 16 test groups (3 settings, 2 animal species, 3 sample types - excluding sheep milk samples) assuming an average prevalence of 8%\(^6\). This testing approach would yield a total of at least 144 *Brucella* positive samples, through testing of 1,824 samples. However, by utilising serological and clinical history data available through BACZOO, we will be able to target our PCR testing at higher risk samples (e.g. from herds/villages with high seroprevalence and/or history of abortion). This targeted testing will enable detection of more than the 144 positives anticipated with random testing, up to a total of 555 positives expected in the total samples that will be collected (Table 1). The Bruceladder multiplex PCR\(^{28,33}\) will be established at KCMC to enable species-typing of *Brucella* positive samples. Assuming that 20% of *Brucella* PCR positive samples can be typed with this test, this approach will yield data on approximately 29-111 typed *Brucella* samples (from 144-555 samples). Aliquots of *Brucella* PCR positive milk and swab samples will also be shipped to the AHVLA to attempt culture of isolates and species-typing using Bruceladder and/or SNP typing\(^{34}\). DNA extracts from any *Brucella* PCR positive samples and a subset of negative DNA extracts will also be shipped for cross-validation of the detection and typing PCRs established at KCMC.

**Research objective 2: Active human case finding.** To determine which *Brucella* species are responsible for human illness it is necessary to collect diagnostic samples from human cases that can be typed. The identification and sampling of active human brucellosis cases is challenging but surveillance approaches targeting febrile patients have been successful at other sites\(^{35,36}\) and disease risk and impacts are highest in marginalised livestock-keeping communities\(^{37}\), so we will conduct targeted sampling of these high risk human groups in Tanzania. Data gathered from the district hospital in Endulen, northern Tanzania over the period 1998-2008 reveal an average of 162 diagnosed *Brucella* cases per year, with an annual incidence of 125 cases/100,000 person years.
(unpublished data based on a clinical history and serological test results). This incidence estimate is in line with some of the highest *Brucella* incidence estimates reported from other countries.\textsuperscript{38} Like other incidence estimates based on hospital based surveillance, this is likely to be an underestimate of the true incidence (e.g. by 12-18 times).\textsuperscript{36} We will extend febrile illness surveillance approaches established at KCMC and train additional staff to apply these techniques at healthcare facilities located in higher risk brucellosis areas.

We will recruit two phlebotomists, one based at Endulen Hospital and one at the FAME clinic in Karatu, which also serves a pastoralist population and is recognised locally as a reporting centre for possible brucellosis patients. Approximately 10 patients are diagnosed as *Brucella* cases each month at the Endulen Hospital. Based on the assumptions that similar case numbers are seen at FAME and that <50% of patients with clinical signs consistent with brucellosis (e.g., fever and additional non-specific signs) are likely to test positive for *Brucella* antibodies, we expect to enrol at least 20 patients per month into this study from each clinic. Over 9 months, this will give us a sample of 360 patients, sufficient to be 95% confident that at least 6 will be *Brucella* blood culture positive, given an estimated prevalence of 3% (based on *Brucella* culture rates from studies of febrile populations conducted in Egypt\textsuperscript{35,36}). However, culture positivity rates of 23%\textsuperscript{39} and 74%\textsuperscript{40} have been reported from groups of symptomatic seropositive patients. If 23% of the 180 probable brucellosis cases likely in this sample were culture positive this would yield 41 *Brucella* isolates.

Patients who present with a history of fever within 72 hours or have an objective fever at admission will be eligible for inclusion in the study. Up to 10mL of venous blood will be collected using EDTA blood tubes, inoculated into a BioMerieux BacT/ALERT Standard Aerobic (SA) bottle and transported back to KCMC at ambient temperature within 60 hours. Suspect isolates will be frozen at -86°C, DNA will be extracted at KCMC for *Brucella* confirmation and species-typing there and subcultures will be shipped to AHVLA for confirmatory species-typing using Bruceladder PCR and/or SNP typing. In addition, up to 5mL of venous blood will be collected for serological testing and a questionnaire will be administered to quantify known risk factors for human brucellosis. These data will enable classification of patients using WHO suspected, probable and confirmed brucellosis case definitions, estimation of seroprevalence and identification of brucellosis risk factors in this high-risk pastoralist population.

Research objective 3: Source identification. The difficulty of detecting and typing *Brucella* to species level using traditional diagnostic approaches and the consequent sparseness of species-specific *Brucella* data from animals that are linked to human data renders traditional approaches such as simple logistic regression ill-suited for determining the relative importance of different animal hosts in transmission and quantifying risk factors for human brucellosis. The challenge is to complement the relative paucity of species-specific data with the greater volume of serological data to add power to the required analysis. We will construct a hierarchical latent variable model to estimate $Y_{jik}$, the true (unobserved) numbers of the $j$th livestock host (cattle and sheep or goats) associated with the $i$th household that have been exposed to the $k$th *Brucella* spp. and are therefore potential past and present sources for human infection. These estimates are informed by estimating the true (unobserved) numbers of seropositive hosts in each household (from the serological data and associated questionnaire data on household livestock ownership generated through BACZOO) and the proportion of these hosts that have been exposed to either *B. melitensis*, or *B. abortus* (informed by the species-typing data that will be generated through this project). The probability of human seropositivity ($p_{ih}$) (based on human *Brucella* serology data generated through BACZOO) will be modeled in turn as various possible functions of $Y_{jik}$. For example, a simple candidate formulation might be $\logit(p_{ih}) \sim \sum_{j=1}^{J} \sum_{k=1}^{K} \beta_{jk}Y_{jik}$. The credible intervals on the posterior distributions of the $\beta_{jk}$’s enable the risk associated with different possible sources of infection (e.g. cattle infected with *B. abortus*, cattle infected with *B. melitensis*, sheep/goats infected with *B. abortus*, and sheep/goats infected with *B. melitensis*) to be directly evaluated and compared. Different formulations of the risk models can be evaluated using DIC or WAIC. This approach (implemented in JAGS) also provides a natural and powerful framework by which additional domain information related to households (such as herd size, species composition and management practices available through BACZOO household questionnaire data) can be incorporated into the estimation of seroprevalence (see Fig. 1 for an example of a Directed Acyclic
Graph (DAG) that represents a simple form of such a model, and the model hierarchy can be extended to include relevant spatial structure and random effects as appropriate. This analysis should deliver quantified and directly comparable estimates of different risks for human infection that can be used to determine which hosts are the primary sources of human disease and which vaccine should be used in which host species to most effectively minimize human risk. The model will also provide predictions of the expected ratios of *B. melitensis* and *B. abortus* infections in humans that can be examined for consistency with data we anticipate generating directly from human cases.

![Diagram of Brucella seroprevalence](image)

**Fig. 1:** True overall *Brucella* seroprevalence in the *i*th household in the *j*th host type (cattle and sheep/goats) (*Y*(_j_,i)) will be estimated from observed numbers of seropositive animals (*y*(_j_,i)), the number of animals sampled (*n*(_j_,i)) the number of animals owned (*N*(_j_,i)), and relevant domain information (*x*). Likewise, the number of animals seropositive due to the *k*th *Brucella* species (*B. abortus*, and *B. melitensis*) (*Y*(_j_,i,a), *Y*(_j_,i,m)) in each household in each host type will be estimated from observed numbers of animals infected with each species (*y*(_j_,i,a), *y*(_j_,i,m)), the number of animals sampled (*n*(_j_,i,a), *n*(_j_,i,m)), and *x*. Various functions of *Y*(_j_,i,k) can then be used to model the probability of human seropositivity (*p*(_h_,i)) simultaneously estimated from the observed numbers of human seropositive (*y*(_h_,i)) and the number tested (*n*(_h_,i)).

Predicting the statistical power of hierarchical latent variable models is not straightforward. However, we can explore power through simulation using simplified frequentist analogues, and we have used this approach to guide our proposed study design. Results, based on previously published seroprevalence rates, suggest that overall we have a greater than 80% chance of detecting odds ratios of between 2 and 2.5 for simply manifested risk factors. Power will be reduced in peri-urban areas where animal seroprevalence and human sample rates are anticipated to be lower, and here we will depend on power enhancement through combining across zones, and the incorporation of additional domain data.

**Impact Objective:** Formulating control policy. A key ambition of this project is to work with policy makers in Tanzania to ensure that project research outputs are used for the development of brucellosis control policy in Tanzania. International guidelines for developing brucellosis control programmes exist, the WHO have produced guidelines on intersectoral collaboration strategies for control and prevention of brucellosis\(^1\) and FAO have recently published a roadmap for progressive control of brucellosis in animals and humans\(^2\). Both documents emphasise the need for collaboration between human and veterinary health sectors, and argue that an effective one-health surveillance system is an essential prerequisite for any brucellosis control programme. This project strengthens an existing intersectoral research team, provisions this team with important new diagnostic capacity, and brings policy-makers into the research process enabling influence over the research agenda, its outputs and relevance (see Pathways to Impact). This will be achieved through regular project research meetings, project policy meetings convened by Dr. Swai in his
capacity as representative of the Ministry of Livestock and Dr. Shirima in his capacity as head of the Tanzanian Vaccine Institute, and the generation of jointly authored policy-briefs. The project will provide and prepare the evidence-base specifically identified by the Tanzanian government that is required to formulate national brucellosis control policy and place Tanzania on the roadmap for progressive control of this high priority disease.

Programme of work & milestones:
See the attached diagrammatic workplan for more detail on these timelines. 

**Research objective 1 - Pathogen typing:** 1 - PDRA to train in diagnostics at AHVLA (Month(M) 3&7: PDRA, AW and AHVLA lab personnel). 2 - PDRA to set-up diagnostics at KCMC (M4&8: PDRA). 3 - AHVLA lab personnel to travel to Tanzania to troubleshoot and assist PDRA with setting up assays at KCMC (M5&9: PDRA and AHVLA lab personnel). 4 - PCR testing of swab and milk samples (M10-14: PDRA & KCMC lab tech). 5 – Shipment of samples to AHVLA (M15: PDRA). 6 - Culture and subtyping of Brucella positive samples at AHVLA (M16-17: AHVLA staff). 7 - Cross-validation of KCMC PCR results at AHVLA (M17: AHVLA staff). 9 – Data analysis & write-up (M18-20: JH & PDRA).


**Trans-national partnership.** This project involves four Tanzanian and two UK organisations and will result in significant new diagnostic, research and policy development capacity through four transnational partnerships. It will result in two new partnerships between KCMC and TVI/TVLA in Tanzania and the AHVLA in the UK to establish and maintain resilient diagnostic capacity at these Tanzanian institutions. It will strengthen existing links between NMAIST and UG by training a future independent Tanzanian scientist, embedded within a supportive network of international experts. In the medium term it will enable the seeding of future research activities between these organizations. Finally, it will add a new dimension to an existing relationship between MOLDF/TVI and UG to enable ministry officials charged with developing national disease control policy to access a nucleus of experts with a proven track record of translating one-health research into policy.

**Capacity building.** This project will contribute to the enhancement of the long-term scientific capabilities of the southern partners by building the following specific capacities:

**Laboratory infrastructure.** The equipment and expert advice provided through this project will mean that essential Brucella species-typing data can be generated in Tanzania. PCR diagnostics for the detection and species-typing of Brucella will be established at KCMC. Once established, these diagnostics and protocols will be applicable to suspect brucellosis cases in both animal and human populations.

**Skills training.** The NMAIST based PDRA will receive training at the AHVLA in Brucella diagnostics and also gain experience of establishing and validating these techniques in Tanzania. Training in the safe collection, handling and transport of diagnostic specimens for Brucella testing will be provided to study staff as well as others from collaborating institutions such as the Tanzanian Veterinary Laboratories Agency, Tanzanian Vaccine Institute, Sokoine University of Agriculture, Arusha Veterinary Investigation Centre and the Kenyan Zoonotic Diseases Unit.

**Institutional networks.** This project will establish long-term north-south partnerships (described above), facilitating knowledge exchange and strengthening links between UK and Tanzanian partner laboratories, Institutes, and government. The project also supports new south-south
intersectoral links between national veterinary institutions (MoLDF/TVI) and human health-care providers (KCMC, Endulen District Hospital and FAME) and builds one-health surveillance capacity in northern Tanzania. Common protocols for sample collection and transport will be developed through this project so that the members of this collaborative group can share expertise.

**Outputs and outcomes.**

**Research objective 1 - Pathogen typing:** 1 - Establishing new diagnostic capacity to detect *Brucella* and type positives to species level at KCMC. 2 - Training of NMAIST PDRA as well as representatives from other TZ/KE organisations in the use of these tools and techniques. 3- Generation of species-typing data to determine which *Brucella* species are present in different Tanzanian ruminant populations.

**Research objective 2 – Active human case finding:** 1 - Establishing diagnostic capacity to identify active human *Brucella* infections at two health-care facilities serving at-risk pastoralist populations. 2- Generation of species-typing data to determine which *Brucella* species cause human brucellosis in northern Tanzania. 3- Evaluation of the efficacy of a targeted case finding approach to quantify the impacts of brucellosis in high-risk human populations.

**Research objective 3 – Source identification:** 1 - Development of contemporary statistical models to maximize extraction of the maximum information from structured sparse data sets to address fundamental questions about brucellosis transmission. 2 - A ranked set of risk models testing support for different routes by which humans are exposed to brucellosis infection. 3 - Generation of evidence to inform vaccine selection and use for the development of national brucellosis control policy.

**Impact objective – Formulating control policy:** 1 – Production of annual policy briefs. 2 – Provision of the evidence-base required to develop national brucellosis control policy. 3 – Placement of Tanzania on the roadmap for progressive control of this high priority disease.
