# Brucellosis in northern Tanzania:
# Investigating the epidemiology of human infection and evaluating diagnostic test performance in animal hosts

Rebecca F. Bodenham
BSc (Hons), MSc

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Institute of Biodiversity, Animal Health and Comparative Medicine

College of Medical, Veterinary and Life Sciences

University of Glasgow

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# Abstract

Brucellosis is a widespread neglected zoonotic disease. It can cause severe and prolonged illness in people, as well as impacting on animal health and productivity. Brucellosis is endemic in much of sub-Saharan Africa (SSA). The global burden of brucellosis is suspected to be highest in SSA, where there are many livestock-keeping communities. Cattle, sheep and goats are common maintenance hosts of zoonotic *Brucella* spp. Pastoralist communities in frequent contact with these livestock species are at increased risk of infection. This study was performed to improve our understanding of the epidemiology of brucellosis in Tanzania through: a risk factor analysis for human acute brucellosis cases; trialling an active surveillance approach to identify additional cases through household screening in a high-risk population; and latent class analyses to evaluate diagnostic test performance in different animal hosts.

In Chapter 2, questionnaire data were collected from febrile patients attending a rural hospital in the Ngorongoro Conservation Area (NCA), Tanzania. Risk factors associated with acute brucellosis were: having herded cattle, sheep and/or goats in the past 12 months; and decreasing age in years. In Chapter 3, active surveillance in the form of screening household members of febrile patients for exposure to *Brucella* spp. was implemented in the NCA. Screening household members of febrile patients with acute brucellosis led to identification of additional acute cases. However, the study did not find a significant association between the *Brucella* spp. exposure of household members and the household member who sought care at hospital. In Chapter 4, Bayesian latent class analyses were used to evaluate the Rose Bengal plate test (RBT) and the competitive enzyme-linked immunosorbent assay (cELISA) for the diagnosis of livestock brucellosis in northern Tanzania. Sensitivity was variable across livestock models, RBT sensitivity was comparable to cELISA in the bovine model and greater than cELISA in ovine and caprine models. RBT and cELISA specificity was essentially comparable in all livestock models. Conducting parallel RBT and cELISA testing optimised diagnostic test performance in all livestock models.

These novel findings can inform the development and implementation of effective, evidence-based brucellosis prevention and control measures in SSA. Improved knowledge of acute human brucellosis risk factors is important in understanding temporally relevant risks associated with active infection and is a vital tool in developing interventions that prevent transmission. Active surveillance by screening household members requires further study but may prove too resource-intensive for routine implementation in Tanzania. However, it can provide valuable data on disease burden for the population that do not reach a healthcare facility, as well as assist in targeting prevention and control measures towards high-risk populations. In livestock, a parallel RBT and cELISA diagnostic testing approach, potentially implemented at the herd/flock level, would be more effective than using either test alone or serial approaches. Using these data, identification of a national sampling and testing approach can guide the development of a surveillance strategy which is a crucial step towards improving our understanding of brucellosis burden across livestock-keeping settings in Tanzania and wider SSA.

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# Acknowledgements

# Author's declaration

I declare that this thesis is my own original work. The content of this thesis has not been submitted as part of any other degree or professional qualification.

# Abbreviations

| | |
|---|---|
| AGID | Agar Gel Immunodiffusion Test |
| AIC | Akaike Information Criterion |
| aOR | Adjusted Odds Ratio |
| APHA | Animal and Plant Health Agency |
| BCI | Bayesian Credibility Interval |
| BMAT | *Brucella* Microagglutination Test |
| BNH | Brucellosis Negative Febrile Hospital Participant |
| BNHH | Brucellosis Negative Febrile Hospital Participant Household |
| BPAT | Buffered Plate Agglutination Test |
| BPH | Brucellosis Positive Febrile Hospital Participant |
| BPHH | Brucellosis Positive Febrile Hospital Participant Household |
| CDC | Centers for Disease Control and Prevention |
| cELISA | Competitive Enzyme-linked Immunosorbent Assay |
| CFT | Complement Fixation Test |
| CI | Confidence Interval |
| DF | Degrees of Freedom |
| DIC | Deviance Information Criterion |
| ELISA | Enzyme-linked Immunosorbent Assay |
| EMA-*i* | Events Mobile Application |
| FAO | Food and Agriculture Organization of the United Nations |
| FPA | Fluorescence Polarisation Assay |
| GLM | Generalised Linear Model |
| GLMM | Generalised Linear Mixed-effect Model |
| KCRI | Kilimanjaro Clinical Research Institute |
| LCA | Latent Class Analysis |
| LMICs | Low- and Middle-Income Countries |
| LRT | Likelihood Ratio Test |
| MCA | Multiple Correspondence Analysis |
| MCMC | Markov Chain Monte Carlo |
| MDGs | Millennium Development Goals |
| NCA | Ngorongoro Conservation Area |
| NPV | Negative Predictive Value |

| | |
|---|---|
| NTD | Neglected Tropical Disease |
| NZD | Neglected Zoonotic Disease |
| OD | Optical Density |
| OIE | World Organisation for Animal Health |
| OPD | Outpatient Department |
| OPS | *O*-polysaccharide |
| OR | Odds Ratio |
| PCR | Polymerase Chain Reaction |
| PPV | Positive Predictive Value |
| RBT | Rose Bengal Plate Test |
| RIV | Rivanol Test |
| SACIDS | Southern African Centre for Infectious Disease Surveillance |
| SAT | Serum Agglutination Test |
| SD | Standard Deviation |
| SDGs | Sustainable Development Goals |
| SE | Standard Error |
| WHO | World Health Organization |

# Chapter 1 Brucellosis in Tanzania: An introduction to a neglected zoonosis

## 1.1 The neglected zoonoses

In 2000, eight Millennium Development Goals (MDGs) were agreed upon at the United Nations Millennium Summit. One of the eight MDGs was "to combat HIV and AIDS, malaria and other diseases". The designation of "other diseases" included a group termed the neglected tropical diseases (NTDs) (Molyneux et al., 2005). More recently, 2015 brought the classification of 17 Sustainable Development Goals (SDGs), a facet of the 2030 Agenda for Sustainable Development adopted by the United Nations Member States. The third SDG focuses on Good Health and Well-Being, referring to combatting AIDS, malaria and "other diseases" including NTDs by 2030. The NTDs are severe, chronic conditions that are among the most common infections of people living in extreme poverty (Hotez et al., 2007; Hotez and Kamath, 2009), and have been referred to as the "true allies of impoverishment" (Molyneux, 2008). The burden of NTDs is often underestimated, which negatively impacts funding opportunities for their control and research (Maudlin et al., 2009). Of the NTDs, the zoonoses are the most neglected (Molyneux et al., 2011). Zoonoses are defined as diseases that are transmitted between humans and other vertebrates (WHO et al., 2006). Despite being the cause of more than 60% of all infectious disease in humans (Taylor et al., 2001), zoonoses are neglected for four main reasons: lack of robust data on disease burdens in endemic regions; lack of widespread knowledge among clinicians and policy makers concerning zoonotic human disease; lack of laboratory diagnostic capacity; and limited reporting systems that are often fragmented with little communication between public and animal health sectors (Molyneux et al., 2011). Ultimately these issues perpetuate the inability to accurately estimate disease burden and lead to limited disease awareness and political interest in zoonotic disease research and control (Molyneux et al., 2011).

The greatest burden of neglected zoonotic diseases (NZDs) is found in marginalised, impoverished communities (Grace et al., 2012). This is for three key reasons (see Figure 1.1): 1 - an association between people living in poverty

and being in close contact with infected animals and/or consuming infected animal products, meaning that these communities are at high-risk of infection; 2 – these communities are least likely to receive appropriate treatment due to reduced access to healthcare facilities and laboratory diagnostics leading to poor prognosis; 3 – impoverished communities that rely on livestock suffer a double burden from zoonoses as there is an impact on both human health and livestock health and productivity, leading to a greater vulnerability to zoonotic illness (WHO et al., 2006). There is a need for a robust evidence-base estimating the burden of the different NZDs on people and animals, especially in marginalised livestock-keeping communities, so that the importance of the different NZDs is perceived by political and funding bodies (WHO et al., 2006).
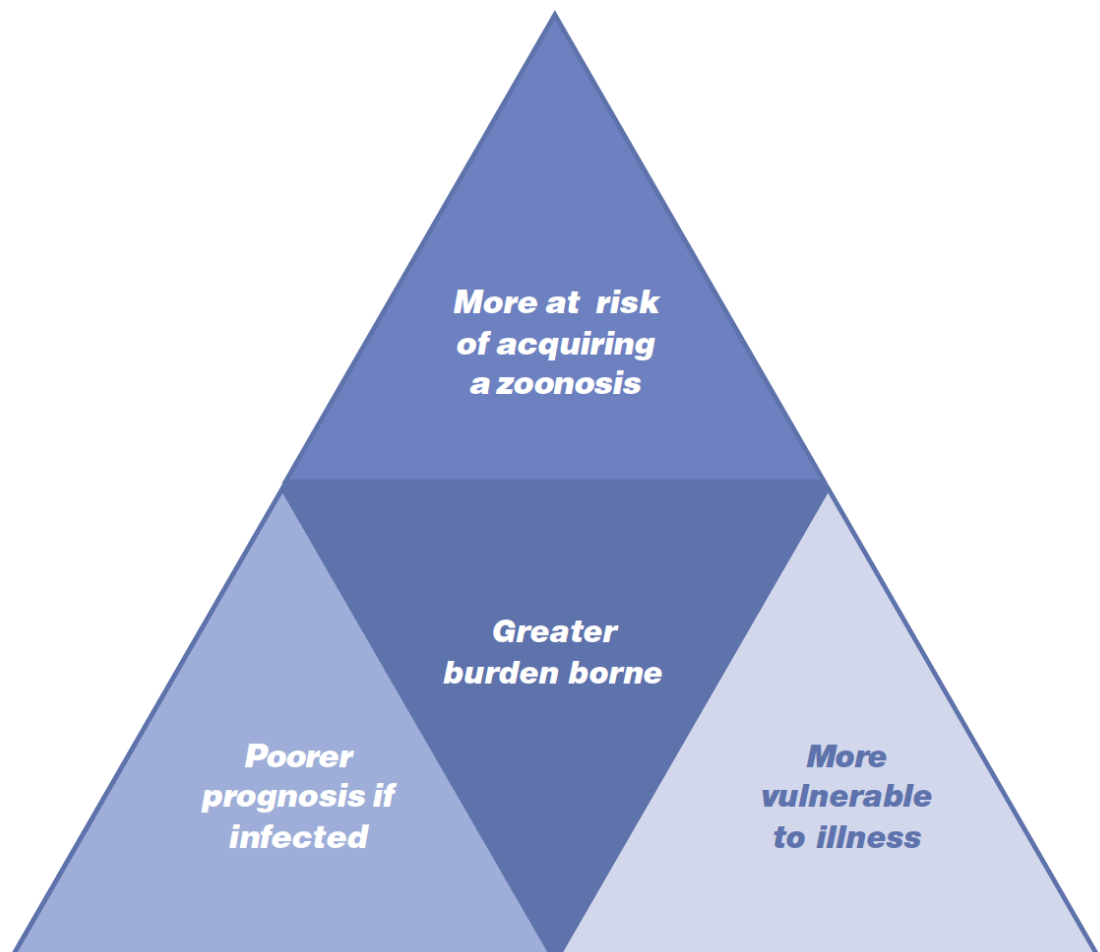


**Figure 1.1: Three key reasons why zoonotic disease burden is greatest for impoverished communities**, *reproduced from WHO et al., 2006*

## 1.2 A short history of brucellosis

Brucellosis is a neglected zoonotic disease that has been recorded in different societies throughout history (Godfroid et al., 2005). In fact, brucellosis may predate *Homo sapiens*, as it has been hypothesised as the cause of skeletal lesions identified in the excavated remains of a 2.4 to 2.8 million year old hominid (*Australopithecus africanus*) found in South Africa (D'Anastasio et al., 2009). During the time of Hippocrates circa 460 BC, a clinical syndrome was described suggestive of brucellosis, including: long-lasting fevers; joint ache; and tumours (Hippocrates, 400BC; Pappas et al., 2008). Vertebral lesions consistent with brucellosis have also been discovered in Roman-era human remains from the volcanic eruption of Mount Vesuvius, 79 AD (Capasso, 1999), as well as the discovery of carbonised cheese with cocco-like forms morphologically and dimensionally similar to the brucellosis causative agent (Capasso, 2002).

In 1887, David Bruce was the first to isolate *Micrococcus melitensis* from the spleen samples of British soldiers in Malta, who were said to have died from undulant fever ((Bruce, 1887), cited by (Rossetti et al., 2017)). A decade later, a veterinarian named L. F. Benhard Bang discovered Bang's bacillus, a bacterium resulting in abortion in cattle and the causative agent of Bang's disease (Bang, 1897). In 1905, Themistocles Zammit isolated *M. melitensis* from goat's milk in Malta, indicating zoonotic transmission of the pathogen ((Zammit, 1905), cited by (Godfroid et al., 2005)). Throughout history, the disease has been referred to by a number of descriptive names such as: intermittent typhoid; Mediterranean fever; Corps disease; and Malta fever (Madkour, 2001). In 1918, microbiologist Alice Evans deciphered the link between Bang's disease and Malta fever, proposing the renaming of the genus to *Brucella* and terming the zoonotic disease "brucellosis" (Evans, 1918; Madkour, 2001; Seleem et al., 2010).

## 1.3 The global burden of brucellosis

Brucellosis is now regarded as the most common zoonosis worldwide (Franco et al., 2007). It is reported across Europe, the Americas, Asia, Australasia and Africa (Pappas et al., 2006). The evolution of the global epidemiology of brucellosis is influenced by a host of anthropogenic factors including: socioeconomic and political change; implementation of control and eradication

programmes; advances in brucellosis detection and reporting systems; illegal importation of infected animals and animal products; and increased international tourism to brucellosis endemic areas (Pappas, 2010; Pappas et al., 2006). Deviation in such factors may help to explain the high variability in incidence of human brucellosis reported between, and even within countries (Dean et al., 2012b).

High-income countries have lower reported brucellosis incidence than low- and middle-income countries (LMICs), although specific low economic status communities in high-income countries can still exhibit an increased brucellosis incidence (Dean et al., 2012b). The World Health Organisation (WHO) estimate the global burden of human brucellosis at 264,073 (95% uncertainty interval: 100,540 - 6,187,148) disability-adjusted life years for 2010 (Havelaar et al., 2015). This is regarded as a conservative estimate that likely underestimates the true disease burden, particularly as robust data on disease incidence and prevalence are scarce for many regions including: Latin America; Asia-Pacific; Eastern Europe; and sub-Saharan Africa (Dean et al., 2012b).

### 1.3.1 Brucellosis in low- to middle-income countries

Brucellosis is considered to be widespread in LMICs (Rubach et al., 2013) and the burden of disease is reported to be large (Doganay and Aygen, 2003; Franco et al., 2007). However, the true burden for both human and animal brucellosis in LMICs is not well quantified (Godfroid et al., 2011). This is especially true concerning impoverished rural communities where robust brucellosis data are generally lacking (Perry et al., 2002). It has been estimated that close to 1 billion impoverished people are involved in livestock-keeping in LMICs (Grace et al., 2012; Staal et al., 2009), and approximately 100-200 million people participate in pastoral production systems (Racloz et al., 2013; Rass, 2006). Pastoralism can be defined as "the use of grassland grazing for the purpose of livestock production" (Racloz et al., 2013). These pastoral production systems can be subset according to the level of mobility in livestock keeping: highly nomadic; transhumant or semi-nomadic; and agro-pastoral (Racloz et al., 2013). Brucellosis is endemic and an important disease in many pastoral systems, causing a high risk of human infection and substantial production losses in

bovine, caprine and ovine populations (McDermott et al., 2013; Mcdermott and Arimi, 2002).

It is important to consider that brucellosis can be present in both rural and urban settings (Racloz et al., 2013). Human contacts with livestock may be less in urban settings as compared to rural, however infection risk is still present through activities such as consumption of raw animal products (Makita et al., 2008). There are now more people that live in close contact with animals than ever before (Plumb et al., 2007). Future projected increases in the human population will result in increased global demand for animal products especially in LMICs (Delgado et al., 2001; Herrero et al., 2012). This means that brucellosis and other zoonoses may increase in prevalence in some populations, particularly those populations with frequent livestock contacts (Plumb et al., 2013) and no enhancements in the livestock production system. This is especially true for marginalised pastoralist communities where increased livestock product demands, coupled with little to no livestock vaccination and minimal hygienic measures may result in increased brucellosis transmission for both livestock-keepers and consumers.

The estimated impact of brucellosis on livestock productivity is not well understood (Roth et al., 2003). However, areas with higher brucellosis prevalences are assumed to have higher productivity losses (McDermott et al., 2013). The economic production impacts of brucellosis in livestock species in LMICs has been reported most frequently for cattle (McDermott et al., 2013). A report from the Food and Agriculture Organization of the United Nations (FAO) has estimated that brucellosis in cattle of SSA reduces milk and meat offtake potential by 5%-10% and 12%-35%, respectively, in traditional extensive production systems and by 4%-7% and 10%-21%, respectively, in smallholder production (Mangen et al., 2002).

## 1.4 Causative agent

The causative agents of brucellosis in humans and animals are Gram-negative, facultative intracellular bacteria of the genus *Brucella* (Godfroid et al., 2005). There are six classical species of *Brucella* including: *B. melitensis*; *B. abortus*; *B.*

*suis*; *B. canis*; *B. ovis*; *B. neotomae*, and six more recently recognised species: *B. ceti*; *B. pinnipedialis*; *B. microti*; *B. inopinata*; *B. papionis*; *and B. vulpis* (Whatmore et al., 2016). A list of identified *Brucella* spp., their associated biovars (i.e. strains of the same *Brucella* species that differ physiologically), preferential hosts and human pathogenicity are given in Table 1.1. The *Brucella* species that most commonly cause human infection are *B. melitensis*, followed by *B. abortus* and *B. suis* (Pappas, 2010). The most virulent *Brucella* spp. are those with domesticated animal hosts (Moreno, 2014). *B. melitensis* is the most virulent form of the disease in humans (Doganay and Aygen, 2003), followed by: *B. suis* biovars 1, 3 and 4; *B. abortus*; and *B. canis* (Moreno, 2014). However,

**Table 1.1: Twelve recognised *Brucella* species, their biovars, preferential hosts and pathogenicity in humans\***

| *Brucella* species | Biovars | Preferential hosts | Human pathogenicity |
|---|---|---|---|
| *B. melitensis* | 1 – 3 | Sheep, Goat | High |
| *B. abortus* | 1 – 6, 9 | Cattle | High |
| *B. suis* | 1, 3 | Pig | High |
| | 2 | Wild boar, Hare | No |
| | 4 | Reindeer, Caribou | High |
| | 5 | Rodents | No |
| *B. canis* | - | Dog | Moderate |
| *B. ovis* | - | Ram | No |
| *B. neotomae* | - | Desert wood rat | No |
| *B. ceti* | - | Cetaceans | Low |
| *B. pinnipedialis* | - | Pinnipeds | Unknown |
| *B. microti* | - | Soil, Vole | Unknown |
| *B. inopinata* | - | Unknown | High |
| *B. papionis* | - | Baboon | Unknown |
| *B. vulpis* | - | Fox | Unknown |

\*Adapted from Godfroid et al., 2011

disease virulence also varies geographically according to the endemic *Brucella* spp. and biovars (Ariza et al., 2007).

The global burden of the different *Brucella* spp. is not well known, although *B. melitensis* and *B. abortus* are considered to cause the majority of human infections (Franco et al., 2007). In the USA the species causing the majority of human infections is reported to be *B. melitensis*, thought to be largely caused by the consumption of imported contaminated dairy products, *B. abortus* is less prevalent (Pappas et al., 2006). In western Europe, human brucellosis is mainly constrained to the Mediterranean basin (Pappas et al., 2006). In countries such as Greece and Spain, reported human brucellosis cases are largely caused by *B. melitensis* infection (Taleski et al., 2002; Valdezate et al., 2007). In many LMICs, the human burden of the different *Brucella* spp. is not clear, which is largely due to a lack of capacity to isolate and speciate *Brucella* spp. bacteria (Ducrotoy and Bardosh, 2017). In Egypt, *B. melitensis* and *B. abortus* have been identified by culture in hospital patients, with *B. melitensis* being the predominant species (Jennings et al., 2007). There are few studies in SSA that have successfully speciated the pathogen in humans (Ducrotoy et al., 2017). *B. melitensis* has been identified by culture for hospital patient populations in countries including: Kenya; Somalia; South Africa; and the Gambia (Oomen, 1976; Valenza et al., 2006; Wheat et al., 1995; Wojno et al., 2016) and *B. abortus* in Kenya and Zimbabwe (Bevan, 1930; Oomen, 1976). These examples of both *B. melitensis* and *B. abortus* human infections in SSA suggest that cattle, sheep and goats have a role as livestock maintenance hosts and in the transmission of brucellosis to humans in this region.

## 1.5 Infection in humans

The main transmission routes for human brucellosis are direct contacts with infected animals and their secretions, including via skin abrasion, inhalation, eye conjunctiva or through consumption of infected animal products (Doganay and Aygen, 2003). Examples of human to human transmission are rare and this route of transmission is considered to be negligible (Corbel, 1997). The incubation period can range from days to several months (Robinson, 2003). Mortality rate are low for brucellosis (Grace et al., 2012). However, *Brucella* spp. infection in humans is more severe than in animals (Moreno, 2014). Furthermore, human brucellosis has been described as "the disease that rarely kills anyone, but often makes a patient wish they were dead" (Seleem et al., 2010), and "major among

the zoonoses in the illness and misery it causes" (Meyer, 1954). The clinical manifestations of brucellosis are variable and non-distinct, meaning clinical diagnosis alone is inaccurate (Rubach et al., 2013). Acute illness may include fluctuating fever (Aygen et al., 2002), as well as other debilitating conditions such as myalgia, arthralgia and back pain (Dean et al., 2012a). *Brucella* spp. infection in women during pregnancy increases the risk of spontaneous miscarriage during the first two gestational trimesters, preterm delivery (birth before 37 weeks of pregnancy), and foetal disease transmission (Arenas-Gamboa et al., 2016). Acute disease may resolve spontaneously (Wright, 1998), or if left untreated, can progress to chronic infection leading to serious complications and permanent sequelae (Corbel, 2006). It is estimated that as many as 30% of undiagnosed acute brucellosis cases may become chronic (Berbari and Wilson, 2001). Epididymo-orchitis has been found to effect 1 in 10 male brucellosis patients, and severe neurological complications and endocarditis have been reported to effect 4 and 1 case per 100 brucellosis patients, respectively (Dean et al., 2012a). Several chronic cases have been reported as lasting more than 30 years (Cutler et al., 2005).

As a febrile illness that lacks differentiating clinical signs, in regions endemic to diseases such as malaria and typhoid fever, brucellosis is at high risk of being misdiagnosed and mistreated (Araj, 2010). In a study of 528 patients clinically diagnosed with malaria in northern Tanzania, only 14 (1.6%) tested positive for malaria, whereas 118 (26.2%) of these patients were positive for a bacterial zoonosis (Crump et al., 2013). In the same study, 16 (3.5%) of 453 patients that underwent laboratory testing were diagnosed with brucellosis (Crump et al., 2013).

## 1.6 Infection in animals

There are many wild and domestic animal natural hosts of the genus *Brucella*. *Brucella spp.* have been identified in a range of wildlife hosts such as: wild boar (*Sus scrofa*); bison (*Bison bison*); elk (*Cervus elaphus*); ibex (*Capra ibex*); African buffalo (*Syncerus caffer*); blue wildebeest (*Connochaetes taurinus*); giraffe (*Giraffa camelopardalis*); and White's tree frog (*Litoria caerulea*) (Alexander et al., 2012; Fyumagwa et al., 2009; Godfroid et al., 2013b; Whatmore et al.,

2015). *B. abortus* and *B. suis* have been isolated from a wide range of wildlife species, whereas isolation of *B. melitensis* is less common (Godfroid et al., 2013b). However, the most important reservoir hosts for human transmission are considered to be cattle, sheep, goats and pigs (Corbel, 2006). The epidemiological characteristics of the different *Brucella* spp. are variable (Godfroid et al., 2005). Broadly, cattle act as the maintenance host for *B. abortus*, sheep and goats are the maintenance hosts for *B. melitensis*, and pigs are the maintenance host for *B. suis* (OIE, 2018). However, understanding the different *Brucella* spp. transmission pathways is complicated by the ability of *Brucella* spp. to spillover into non-preferential hosts, for example *B. suis* and *B. melitensis* can be found in cattle and *B. abortus* in small ruminants (Godfroid et al., 2013a, 2011). Animal to animal transmission of *Brucella* spp. is via: contact with infected aborting animals, aborted foetus or secretions; contact with contaminated grazing areas or animal enclosures following parturition; sexual transmission and artificial insemination from infected animals (Aune et al., 2011; Corbel, 2006; Jergefa et al., 2009; Muma et al., 2006; Osoro et al., 2015). Animal infection is most frequently by ingestion, but may also include transmission by skin abrasion, inhalation, inoculation of conjunctiva or vaginal mucosa (Corbel, 2006; Druett et al., 1956; Stuart et al., 1987; Thorne and Morton, 1978). Clinical manifestation in domestic reservoir hosts is variable and may include: abortion; reduced fertility; carpal hygromas; and chronically lowered milk yields (Grace et al., 2012; WHO et al., 2006). Abortion typically happens during the second half of gestation, and in 75% to 90% of cases abortion occurs once, during the first pregnancy following acute infection (Godfroid et al., 2013b). These variable clinical signs of infection are non-distinctive and therefore diagnosis requires laboratory confirmation (Ducrotoy et al., 2017).

## 1.7 Diagnosis

Both human and animal brucellosis should be confirmed by laboratory diagnostics. There are a range of tests available that can be defined as either: 'direct' in that they attempt to detect the presence of *Brucella* bacteria in the sample; or 'indirect' whereby the test detects the host response to a *Brucella* spp. infection, such as an antibody response (Ducrotoy and Bardosh, 2017;

Godfroid et al., 2010). Each testing approach has its own advantages and disadvantages.

### 1.7.1 Direct diagnostic tests

The gold standard laboratory method for diagnosis of definitive *Brucella* spp. infection in humans and animals is isolation of the bacterium by culture. Blood is the most common culture sample in human brucellosis, whereas blood, vaginal fluid, parturition products and milk are common culture samples in animal brucellosis (Corbel, 2006). In humans, the isolation success rate can be up to 86% during periods of fever, however during periods of no fever or if antibiotics have been administered, the success rate can be low (Ducrotoy and Bardosh, 2017). Isolation success for animals can be over 80% (Ducrotoy et al., 2018). However, manipulation of isolated bacteria is slow, expensive and hazardous. *Brucella* spp. infection is one of the most commonly acquired laboratory infections and requires Biosafety Level 3 laboratory facilities (OIE, 2018).

Polymerase chain reaction (PCR) diagnostics have been developed to detect *Brucella* DNA in human and animal samples, such as serum, whole blood and foetal tissue (Al Dahouk et al., 2013; Fekete et al., 1992; OIE, 2018). PCR techniques have a high specificity but are generally regarded as having a lower sensitivity than culture (Godfroid et al., 2010). PCR greatly reduces testing times as compared to culture (Zerva et al., 2001). However, varying protocols and poor test reproducibility complicate the routine application of PCR diagnostics (Ducrotoy and Bardosh, 2017).

Overall, application of culture or PCR techniques are rarely able to be routinely implemented in the LMICs where they are most needed. This is due to: the need for specialised laboratory facilities; unavailability of laboratory technicians with required expertise in implementation and interpretation of results; and the cost per test (Ducrotoy and Bardosh, 2017).

### 1.7.2 Indirect diagnostic tests

The most commonly applied laboratory diagnostic method for brucellosis is serology (Araj, 2010). Brucellosis serology for detection of immunological response to *B. melitensis*, *B. abortus* and *B. suis* infection exploits the fact that

the cell surfaces contain *O*-polysaccharide (OPS) (Nielsen, 2002). Evidence for exposure to OPS can then be identified by tests based on either whole cell antigen or smooth lipopolysaccharide preparation for these three *Brucella* spp. (Nielsen, 2002). However, in the detection of other important species such as *B. canis* and *B. ovis* rough lipopolysaccharide specific serology must be used, as the immune response specific to these *Brucella* spp. means that they do not cross-react with smooth lipopolysaccharide tests (Araj, 2010).

Human and animal serological tests can be broken down into the following classifications: agglutination tests; primary binding assays; precipitation tests; and complement fixation tests (Nielsen, 2002). The serum agglutination test (SAT) is a well validated, common reference test in the diagnosis of human brucellosis (Al Dahouk et al., 2013; Araj, 2010). In cattle, sheep and goats the Rose Bengal plate test (RBT) is often used as a screening test, and requires confirmation by an additional serological test such as a complement fixation test (CFT) or an enzyme-linked immunosorbent assay (ELISA) (Godfroid et al., 2013a). There are a wide range of diagnostic tests that have been developed for brucellosis (Moreno, 2014), with different degrees of test validation data available for various study species and populations. Serological tests suitable for the detection of human and animal brucellosis, as defined by WHO and OIE, are given in Table 1.2.

Serological tests are relatively fast to perform and for the most part require minimal equipment, making serology the most commonly applied technique in brucellosis endemic areas (de Glanville et al., 2017). However, it is important to consider some of the limitations of serology also. One such example is that the OPS cell surface found in some of the key zoonotic *Brucella* spp. is similar to that of other bacteria such as *Yersinia enterocolitica* 0:9, resulting in reduced test specificity due to the potential for false positive results given by test cross-reactivity (Kittelberger et al., 1995). There is also no one recommended serological test and no standardised reference antigen, consequently the source of the antigen used can affect the test result (Araj, 2010). Additionally, serological tests cannot identify *Brucella* to the species-level (Godfroid et al., 2013a).

**Table 1.2: Human and animal brucellosis suitable serological tests and test type classifications***

| Serological test | Test classification | Human brucellosis (WHO) | Animal brucellosis (OIE) |
|---|---|---|---|
| Rose Bengal plate test (RBT) | Agglutination | ✓ | ✓ |
| Serum agglutination test (SAT) | Agglutination | ✓ | ✓ |
| Coombs antiglobulin test | Agglutination | ✓ | |
| Buffered plate agglutination test (BPAT) | Agglutination | | ✓ |
| Enzyme-linked immunosorbent assay (ELISA) | Primary binding assay | ✓ | ✓ |
| Fluorescence polarisation assay (FPA) | Primary binding assay | | ✓ |
| Complement fixation test (CFT) | Complement fixation | ✓ | ✓ |

*Adapted from Corbel et al., 2006 & OIE, 2018

## 1.7.3 Active brucellosis versus *Brucella* spp. exposure

The various categories of brucellosis diagnostic tests mentioned above differ in their ability to detect active infection versus evidence of *Brucella* spp. exposure. In the case of culture, a positive culture result can be interpreted as definitive evidence of an active brucellosis infection, acute or chronic (Mantur et al., 2008). A PCR positive on the other hand shows evidence of *Brucella* spp. DNA presence only. This makes clinical interpretation of PCR results difficult, as active and historic infections are not easily distinguished and persistence of *Brucella* spp. DNA is variable and not well understood (Al Dahouk et al., 2013). The interpretation of serology is also complex, in that a seropositive result is not able to differentiate between active infection and historic exposure to *Brucella* spp., unless antibody titres are quantified (Al Dahouk and Nöckler, 2011). Additionally, it is possible to show an antibody response to *Brucella* spp. exposure without manifestation of clinical signs of infection (Zhen et al., 2013).

The ability to differentiate between active infection versus evidence of historic exposure is especially important when diagnosing and treating human brucellosis. The Centers for Disease Control and Prevention (CDC) have defined

the criteria required for the identification of both probable and confirmed acute human brucellosis (CDC, 2010). The CDC case definition for *probable* acute human brucellosis is "a clinically compatible illness, with a *Brucella* total antibody titre of ≥160 by SAT or BMAT in at least one serum sample, or detection of *Brucella* DNA in a clinical specimen by PCR". The CDC case definition for *confirmed* acute brucellosis is "a clinically compatible illness, with culture and identification of the *Brucella* spp., or evidence of a four-fold or greater rise in *Brucella* antibody titre between acute- and convalescent-phase sera obtained ≥2 weeks apart" (CDC, 2010).

## 1.8 Treatment

Treatment of human brucellosis is by dual antibiotic therapy. WHO recommendations for the treatment of uncomplicated brucellosis in adults and children over seven years old is primarily by: a tetracycline, ideally doxycycline 100 mg orally every 12 hours for six weeks; plus an amino-glycoside such as, streptomycin 1 g intramuscularly per day for two to three weeks, or gentamicin 5 mg per kg intravenously or intramuscularly per day for seven to ten days (Corbel, 2006). In children aged seven years and below, the recommended treatment is primarily by: trimethoprim/sulfamethoxazole 8/40 mg per kg orally every 12 hours for six weeks; plus streptomycin 30 mg per kg intramuscularly per day for three weeks, or gentamicin 5 mg per kg intravenously or intramuscularly per day for seven to ten days (Corbel, 2006).

The treatment of brucellosis is non-trivial and protracted. Treatment with doxycycline and amino-glycosides can cause adverse effects such as abdominal pain and light sensitivity (Roushan et al., 2006). Intravenous or intramuscular administration of amino-glycosides require repeat visits to a healthcare facility. This means that in geographically remote areas, up to a three-week inpatient admission is required. Little to nothing is known about patient compliance with brucellosis treatment (Pappas et al., 2005), such as completion of treatment rates for orally administered doxycycline over extended periods.

## 1.9 Brucellosis in pastoral sub-Saharan Africa

The population of sub-Saharan Africa (SSA) was estimated at over 1.07 billion people in 2018 (The World Bank, 2019). Brucellosis is endemic across SSA, and is a major threat to the region (Moreno, 2014). Much of the global burden of brucellosis is found in SSA (Racloz et al., 2013), particularly in countries with extensive pastoral areas (Njeru et al., 2016b). At least 50 million people in SSA are estimated to be involved in pastoralism (Rass, 2006). East Africa holds the highest density of livestock kept in pastoral and agro-pastoral systems (Rass, 2006). The persistence of brucellosis in these pastoral areas is due to factors such as: reduced access to public services; small and dispersed human populations; large distances; severe environmental conditions; insufficient governance; multi-species herd/flock composition; and limited regional epidemiological knowledge (Plumb et al., 2013; Racloz et al., 2013).

## 1.9.1 Human brucellosis in Tanzania

Human brucellosis seroprevalence estimates have varied widely, from 0.0% to 36.5% in Tanzania (Assenga et al., 2015; Bouley et al., 2012; Carugati et al., 2018; Chipwaza et al., 2015; Crump et al., 2013; Kunda et al., 2007; Orsel et al., 2015; Shirima et al., 2010; Shirima and Kunda, 2016; Swai and Schoonman, 2009). Various recent studies in wider East Africa, estimate human brucellosis seroprevalence between 1.3% and 17.0% (Kiambi, 2012; Migisha et al., 2018; Miller et al., 2016; Nakeel et al., 2016; Nanyende, 2010; Njeru et al., 2016a; Omballa et al., 2016; Osoro et al., 2015; Tumwine et al., 2015). Variation in seroprevalence estimates across studies can be explained by a number of factors including varied study design, study setting and study population, diagnostic tests used and brucellosis case definition. For a comparison of the different human brucellosis study details in Tanzania see Table 1.3.

Studies investigating febrile hospital patient populations have reported seroprevalence estimates between 2.9% and 36.5% in Tanzania (Bouley et al., 2012; Carugati et al., 2018; Chipwaza et al., 2015; Orsel et al., 2015). Of those hospital-based febrile surveillance studies, the studies that have identified acute brucellosis using the CDC case definition have estimated seroprevalence of confirmed acute brucellosis to range from 2.9% to 3.5%

**Table 1.3: Summary of human brucellosis seroprevalence studies in Tanzania,** *RBT is Rose Bengal plate test, BPAT is buffered plate agglutination test, cELISA is competitive enzyme-linked immunosorbent assay, Riv is rivanol precipitation test, BMAT is Brucella microagglutination test*

| Study area | Study population | Study design | Sample size | Study population seroprevalence (%) | Diagnostic test | CDC-defined brucellosis case status | Reference |
|---|---|---|---|---|---|---|---|
| Pastoral & agro-pastoral, northern Tanzania | Patients with: fever; headache; arthralgia; malaise; backache; or anorexia | Cross-sectional | 1586 | 6.2 | cELISA | Exposure | (Kunda et al., 2007) |
| Urban & rural, northern Tanzania | High-risk occupational groups | Cross-sectional | 199 | 5.5 | RBT | Exposure | (Swai and Schoonman, 2009) |
| Pastoral & agro-pastoral, northern Tanzania | Pastoralist & agro-pastoralist households | Cross-sectional | 460 | 8.3 | cELISA | Exposure | (Shirima et al., 2010) |
| Urban, northern Tanzania | Febrile patients | Prospective cohort | 454 | 3.5 | BMAT | Confirmed acute | (Bouley et al., 2012) |
| Agro-pastoral, western Tanzania | Agro-pastoralist households | Cross-sectional | 340 | 0.6 <br> 0.6 | RBT & BPAT <br> RBT & Riv | Exposure | (Assenga et al., 2015) |
| Agro-pastoral, central-eastern Tanzania | Febrile patients aged 2-13 years | Cross-sectional | 370 | 7.0 <br> 11.4 | IgM-ELISA <br> IgG-ELISA | Exposure | (Chipwaza et al., 2015) |
| Pastoral, northern Tanzania | Febrile and/or suspected malaria patients | Cross-sectional | 159 | 5.7 <br> 36.5 | Slide card agglutination <br> IgM-ELISA & IgG-ELISA | Exposure | (Orsel et al., 2015) |
| Agro-pastoral, northern Tanzania | Agro-pastoralist households | Cross-sectional | 82 | 0.0 | RBT or cELISA | Exposure | (Shirima and Kunda, 2016) |
| Urban, northern Tanzania | Febrile patients | Prospective cohort | 1095 | 2.9 | BMAT | Confirmed acute | (Carugati et al., 2018) |

(Bouley et al., 2012; Carugati et al., 2018). All other studies of human brucellosis in Tanzania (see Table 1.3) can be classified as evidence of exposure to *Brucella* spp. (past or present), due to choice of diagnostic test. A febrile surveillance study at a rural hospital in Arusha Region estimated seroprevalence at 36.5% using IgM and IgG ELISA and 7.0% using a slide card agglutination test (Orsel et al., 2015). This study was conducted in a semi-nomadic pastoral area where it is expected that brucellosis is endemic (Njeru et al., 2016b). However, the difference in seroprevalence estimates between tests from the same study is non-negligible, which highlights the importance of the application of standardised diagnostic tests and test antigens in generating population representative seroprevalence estimates. Lack of comparability across studies and variation in prevalence estimates complicates the already difficult task of understanding the true burden of human brucellosis in Tanzania and across LMICs. Examples of febrile hospital-based studies in East Africa that meet the CDC acute brucellosis case definition are few. The available East African studies estimate acute brucellosis prevalence of febrile hospital patients to range between 4.3% and 15.4% (Kiambi, 2012; Migisha et al., 2018; Njeru et al., 2016a). In comparison, it would appear that studies in Tanzania have a comparatively low acute brucellosis detection rate. However, study design must again be considered, such as the study definition for a brucellosis case. The acute brucellosis estimate range for East Africa includes studies using both probable and confirmed acute brucellosis to define a case, which may explain some of the variation as compared to the estimate range for Tanzania, which has used evidence of confirmed acute brucellosis only.

### 1.9.2 Livestock brucellosis in Tanzania

Livestock brucellosis seroprevalence studies have been conducted in Tanzania across a range of livestock-keeping systems, see Table 1.4 for study descriptions. Seroprevalence has been reported to range from 3.0% to 18.0% in cattle, 0.0% to 23.1% in sheep and 0.0% to 13.8% in goats (Assenga et al., 2015; Chitupila et al., 2015; Jiwa et al., 1996; John et al., 2010; Lyimo, 2013; Mathew et al., 2015; Mellau et al., 2009; Sagamiko et al., 2018; Shirima et al., 2010; Shirima and Kunda, 2016; Swai and Schoonman, 2010; Weinhaupl et al., 2000). As in humans, these variable livestock seroprevalence estimates are likely influenced by a

**Table 1.4: Summary of livestock brucellosis seroprevalence studies in Tanzania**, *RBT is Rose Bengal plate test, cELISA is competitive enzyme-linked immunosorbent assay, BMAT is Brucella microagglutination test, iELISA is indirect enzyme-linked immunosorbent assay, and SAT is serum agglutination test*

| Location in Tanzania | Livestock-keeping system | Sampling | Species | Sample size | Seroprevalence (%) | Diagnostic tests | Reference |
|---|---|---|---|---|---|---|---|
| Northern | Commercial | Cross-sectional | Cattle | 13078 | 10.8 | SAT | (Jiwa et al., 1996) |
| Eastern | Pastoral & smallholder | Cross-sectional | Cattle | 2563 | 12.5 | SAT | (Weinhaupl et al., 2000) |
| Northern | Pastoral | Cross-sectional | Cattle | 200 | 10.0, 6.0 | RBT, BMAT | (Mellau et al., 2009) |
| | | | Goats | 87 | 11.5, 13.8 | RBT, BMAT | |
| | | | Sheep | 13 | 7.7, 23.1 | RBT, BMAT | |
| Northern | Pastoral & agro-pastoral | Matched case-control | Cattle | Unknown | 3.0 | RBT & cELISA | (John et al., 2010) |
| | | | Goats | Unknown | 4.6 | RBT & cELISA | |
| | | | Sheep | Unknown | 3.4 | RBT & cELISA | |
| Northern | Pastoral & agro-pastoral | Cross-sectional | Cattle | Unknown | 4.9 | cELISA | (Shirima et al., 2010) |
| | | | Small ruminants | Unknown Total=2723 | 6.5 | cELISA | |
| Northern | Pastoral | Cross-sectional | Cattle | 246 | 7.3 | RBT | (Swai and Schoonman, 2010) |
| | Smallholder | Cross-sectional | Cattle | 409 | 4.1 | RBT | |
| Eastern | Smallholder | Cross-sectional | Cattle | 450 | 18.4 | cELISA | (Lyimo, 2013) |
| Western | Agro-pastoral | Cross-sectional | Cattle | 1103 | 6.8 | RBT & cELISA | (Assenga et al., 2015) |
| | | | Goats | 248 | 1.6 | RBT & cELISA | |
| Western | Agro-pastoral | Cross-sectional | Cattle | 410 | 5.6 | RBT & cELISA | (Chitupila et al., 2015) |
| Southern | Commercial | Cross-sectional | Cattle | 200 | 18.0 | RBT & iELISA | (Mathew et al., 2015) |
| | | | Goats | 50 | 2.0 | iELISA | |
| | | | Sheep | 35 | 5.7 | iELISA | |
| Northern | Agro-pastoral | Cross-sectional | Cattle | 288 | 5.6 | RBT & cELISA | (Shirima and Kunda, 2016) |
| | | | Small ruminants | 125 | 0.0 | RBT & cELISA | |
| Southern | Agro-pastoral & commercial | Cross-sectional | Cattle | 1211 | 9.3 | RBT & cELISA | (Sagamiko et al., 2018) |

number of factors including the different study populations, the use of different diagnostic tests and even variability in the quality of the test antigen used where the same or a similar test has been used across different studies. Seroprevalence estimates generated using OIE recommended tests for livestock populations in East Africa are: 3.5% to 21.9% in cattle; 7.3% to 8.6% in sheep; and 2.0% to 17.0% in goats (Makita et al., 2011b; Miller et al., 2016; Nakeel et al., 2016; Nanyende, 2010; Osoro et al., 2015). *Brucella* spp. seroprevalence estimates for cattle and goat species in Tanzania to date do not differ greatly from estimates reported for wider East Africa. However, in sheep seroprevalence has been estimated to be higher in Tanzania than for other studies of East Africa (Mellau et al., 2009). This estimate may be a true representation of the prevalence of brucellosis in the pastoral study site. Equally, the elevated estimate may be a consequence of study design. Two diagnostic tests were run in parallel in the study, the RBT estimated a seroprevalence three times lower than the 23.1% seroprevalence estimated by the BMAT (Mellau et al., 2009). This variation in seroprevalence in the same study is an argument for the use of both a screening and confirmatory or complementary diagnostic test in order to generate more robust seroprevalence estimates.

## 1.10 Control strategies

In order to control human brucellosis, it is necessary to identify and control the *Brucella* spp. found in the animal reservoir hosts (Bamaiyi, 2016; Seleem et al., 2010). Therefore, control programmes in high-income countries largely focus on animal and livestock-keeper interventions, which have resulted in reduced animal incidence and few reported human cases (Seleem et al., 2010; WHO et al., 2006). These animal and livestock-keeper interventions can include: adequate vaccination of susceptible animals; use of suitable brucellosis diagnostics; control of livestock movements; test and slaughter of infected animals; livestock-keeper compensation for culled animals; and certification and financial incentives for disease-free herd status (Moreno, 2014; Saegerman et al., 2010).

Human brucellosis is controlled, with only sporadic infections, across North America, Australia, New Zealand and parts of northern Europe, including the UK (Cutler et al., 2005; Moreno, 2014). Countries achieving control or even eradication of brucellosis are still susceptible to disease re-emergence due to livestock movements (Cutler et al., 2005). Therefore, ongoing surveillance, including abortion reporting, as well as pre- and post-import testing is important, although can be difficult to maintain when incidence is low (England et al., 2004; Maudlin et al., 2009).

Zoonotic disease control becomes more complicated in areas where there is an interface with wildlife hosts (Grace et al., 2012). A well-known example of this is in the Greater Yellowstone Ecosystem where elk and bison represent the last reservoir hosts for *B. abortus* in the USA, which results in periodic reinfection in livestock hosts (Scurlock and Edwards, 2010).

## 1.10.1 Control in sub-Saharan Africa

In SSA, brucellosis control has been infrequently attempted outside southern Africa (Mcdermott and Arimi, 2002; OIE, 2013). The approaches that have been successful for brucellosis control in high-income countries are not necessarily the approaches that will work in SSA. Control programmes in these settings require infrastructure such as: capacity building in the form of educating communities about brucellosis risks; active surveillance and reporting; and sufficient laboratory facilities, effective diagnostic tests and trained technicians (Seleem et al., 2010).

Furthermore, test and slaughter of livestock, one of the key control approaches in high-income countries, cannot be considered a realistic approach in SSA (WHO et al., 2006). Firstly, because the resources to compensate livestock-keepers for culled animals are not available, which would severely impair compliance with such a strategy. Secondly, there are more than 165 million impoverished people participating in some form of livestock-keeping in SSA (Grace et al., 2012; Herrero et al., 2012), and these livestock-keepers are often dependent on their animals not only as a source of income but also as an important source of nutrition (Rubach et al., 2013). Convincing these communities to have

potentially asymptomatic animals slaughtered, as well as to not consume the meat from these animals would be extremely difficult to implement and regulate.

Restricting and monitoring livestock movements is another key aspect of brucellosis control that is challenging to implement in SSA. This is particularly true for potentially high-brucellosis risk nomadic communities that move in remote areas and may cross international borders (Corbel, 2006). There is a risk that livestock movements could even increase due to the introduction of a brucellosis control strategy, where testing animals for brucellosis can lead to the distress sale (i.e. urgent sale often at a compromised price) of test-positive livestock (Renukaradhya et al., 2002).

Vaccination of livestock is viewed as a feasible approach to the control of brucellosis in SSA (WHO et al., 2006). Before a vaccination campaign can be developed, the *Brucella* species causing human infections must be identified, so that the correct animal host species can be targeted for vaccination. However, characterised isolates for human infections in SSA are not common (Ducrotoy et al., 2017). Representative data on the true burden of brucellosis is also important in guiding vaccination campaign decision making. Additionally, the implementation of a sustainable approach to vaccination must be carefully considered, as an approach that is not maintained successfully can have serious consequences for both public health and livestock production (Godfroid et al., 2013a). This has been demonstrated in Greece, where successful national vaccination of small ruminants using Rev-1 was discontinued (Minas et al., 2004). This was followed by a rapid increase in livestock and human brucellosis incidence which required implementation of an emergency mass vaccination campaign (Minas et al., 2004). If sufficient evidence about the epidemiology of brucellosis in an area can be collected and sustainable funding can be allocated to a vaccination campaign, then additional aspects of vaccination need to be carefully considered. These aspects include: the demographic group to be targeted; type of vaccine to use; the route of vaccination (conjunctival or subcutaneous); and the frequency of vaccination (Ducrotoy et al., 2017).

An additional approach in brucellosis control that has been endorsed for SSA is the implementation of specific hygiene measures, such as the pasteurisation of dairy products (Doganay and Aygen, 2003). The aim of improved hygiene measures is to reduce exposures to infected animals and their products (Corbel, 2006). Effecting change in traditional consumption and livestock-keeping practices of local communities will likely be extremely challenging. Therefore, control strategies that are developed considering the specific needs and perceptions of the community are vital (Marcotty et al., 2009).

Finally, due to the zoonotic nature of brucellosis a One Health approach must be taken with regard to its control (Hattendorf et al., 2017). The control of brucellosis is a complex task involving a wide range of stakeholders. There must be an inclusive and holistic multi-sectoral approach to any brucellosis control intervention to maximise its impact (Godfroid et al., 2013a).

## 1.11 Overview of thesis aims

Brucellosis is an epidemiologically complex disease that causes incapacitating long-lasting illness and diminishes livestock productivity. For impoverished communities in LMICs, without the control of NZDs such as brucellosis the cycle of disease and poverty will continue (Molyneux et al., 2011). In SSA where the global brucellosis burden is estimated to be greatest (Racloz et al., 2013), the availability of robust data is crucial in understanding the true burden of the disease (Dean et al., 2012b). Novel representative data on brucellosis burden can be used to inform the development of effective evidence-based disease prevention and control strategies (Corbel, 2006).

The broad aim of this thesis is to improve our understanding of identified knowledge gaps in the epidemiology of brucellosis in Tanzania and wider SSA. The knowledge gap that Chapter 2 aims to address is the need for more detailed epidemiological data, particularly in areas suspected to be at high risk of brucellosis in Tanzania. This chapter describes identification of the risk factors associated with acute human brucellosis in a pastoralist community of Tanzania. Representative data on acute brucellosis risk factors can help to identify the demographic at highest risk of recent active infection, which is vital in the

effective prioritisation of disease control interventions. This is the first study of risk factors for acute brucellosis in a pastoralist community in Tanzania, and one of few in East Africa. The knowledge gap that Chapter 3 aims to address is the need for surveillance approaches that assist in providing a true representation of brucellosis burden in Tanzania. This chapter describes investigation into the use of community-based active surveillance in the form of screening household members of febrile hospital patients in a pastoralist community. It is recognised that only a proportion of the population in SSA will access a healthcare facility when suffering from febrile illness (Panzner et al., 2016). Therefore, community-based approaches can play a very important role in brucellosis surveillance. The key aims of this study are to determine if additional acute brucellosis cases can be identified in household members of febrile patients and to evaluate if there is any evidence of grouping of *Brucella* spp. exposure status between household members and febrile hospital patients. This is the first study to implement this form of active surveillance for brucellosis in Tanzania. Similar studies in SSA are not evident. The knowledge gap that Chapter 4 aims to address is the need for a validated and standardised brucellosis diagnostic test approach in Tanzania. This chapter describes evaluation of brucellosis diagnostic test performance in cattle, sheep and goats, as well as estimating disease prevalence in different livestock-keeping communities. In the absence of a gold standard test, diagnostic test performance data are vital in evaluating the probability of available brucellosis tests to correctly identify *Brucella* spp. exposures. The key aim of this study is to generate robust estimates for individual test performance and testing protocols in cattle, sheep and goats. This is the first study to evaluate RBT and cELISA diagnostic test performance in Tanzania for cattle, it is likely the first study in SSA for sheep and goats. The results from each of these chapters can be used to improve estimates of the true burden of brucellosis in SSA and inform the implementation of an evidence-based brucellosis prevention and control strategy for Tanzania.

# Chapter 2 Risk factors for acute brucellosis in febrile patients from a pastoralist community

## 2.1 Introduction

Understanding risk factors for human brucellosis is invaluable in the development of successful brucellosis prevention and control interventions, and in effective policy-making (Dean et al., 2012a). Specifically, risk factor data can be used to feedback to the study community. This enables communication about high risk transmission activities, as well as methods to decrease transmission, therefore contributing towards reducing the impacts of human brucellosis (Corbel, 2006).

In the identification of risk factors for human brucellosis, different definitions for human brucellosis status are frequently investigated. Often, reported risk factors for human brucellosis are defined by serology, this is because serology is an easier and safer diagnostic approach as compared to the gold standard method of bacterial isolation by culture (Díaz et al., 2011). However, when conducting brucellosis serology without quantifying antibody titres, it is not possible to distinguish the stage of infection (Al Dahouk et al., 2013), or indeed identify active infection from historic *Brucella* spp. exposure (Al Dahouk and Nöckler, 2011). This is because *Brucella* spp. antibodies can persist in the blood for years following recovery (Araj, 2010). Serology tests that identify a single high antibody titre or a four-fold increase in titre between acute and convalescent-phase sera are often classified as active brucellosis (Al Dahouk et al., 2013). Identifying risk factors for a population with acute brucellosis

infection will likely identify more temporally relevant risk activities than investigating risk factors for exposure to *Brucella* spp. If risk factors identified for acute brucellosis infection are used to guide disease prevention and control interventions, they may be more effective in impacting on *Brucella* spp. transmission than those risk factors determined by *Brucella* spp. exposure.

Across high- to low-income countries, the risk factors for human brucellosis exposure broadly include, consumption of unpasteurised dairy products and direct contact with infected animals (Dean et al., 2012a; Rubach et al., 2013). However, risk factors for human brucellosis are setting-specific, and are determined by the activities of the human population under consideration and the host species present (Cash-Goldwasser et al., 2018). Focusing within East Africa (including Tanzania, Kenya, Uganda, Burundi and Rwanda), the risk factors for human brucellosis exposure can usually be classified within the following groups: ingestion of raw animal products (such as meat, blood and milk); close contact with livestock (such as assisting in animal parturition); slaughtering, cleaning and handling carcasses; occupation (which is often a proxy for some of the other frequently reported risk behaviours); and a reported family history of brucellosis (John et al., 2010; Migisha et al., 2018; Miller et al., 2016; Nanyende, 2010; Nasinyama et al., 2014; Njeru et al., 2016b; Orsel et al., 2015; Osoro et al., 2015; Qido, 2008; Rujeni and Mbanzamihigo, 2014; Swai and Schoonman, 2009; Tumwine et al., 2015).

Pastoralist communities in particular are in frequent contact with livestock, and in endemic areas are at a high risk of human brucellosis infection (Mcdermott and Arimi, 2002; Rubach et al., 2013). In sub-Saharan Africa (SSA), 16% of the human population relies on pastoralism (Racloz et al., 2013). In Tanzania, approximately 40% of the population are exclusive pastoralists (PINGO's Forum, 2016). The highly mobile nature of pastoralist communities, coupled with low population densities make information gathering challenging, leading to scarcity of epidemiological data for brucellosis (Racloz et al., 2013), as well as for other infectious diseases. Human brucellosis often goes misdiagnosed and uncontrolled in many pastoralist communities (Plumb et al., 2013).

Examples of risk factor analyses for acute brucellosis infection in East Africa are rare. This is because there are few studies that have successfully isolated *Brucella* spp. from culture (Ducrotoy et al., 2017), used PCR confirmation (Doganay and Aygen, 2003), or quantification of serology titres. However, a study including a largely urban population seeking care at hospitals in Moshi, Tanzania, identified assisting in birthing of small ruminants and contact with cattle as risk factors for acute brucellosis infection, whereas consumption of pasteurised dairy products reduced the risk of acute brucellosis (Cash-Goldwasser et al., 2018). In three largely agro-pastoralist communities of Uganda, ingestion of raw dairy products has also been reported as a risk factor for acute infection (Asiimwe et al., 2015). Identification of risk factors for acute brucellosis infection within pastoralist communities are limited. Two Kenyan studies of overlapping pastoralist communities found purchase and consumption of raw dairy products and contact with livestock species to be risk factors for acute brucellosis (Kiambi, 2012; Njeru et al., 2016a). This study is the first to perform a risk factor analysis to identify risk factors for acute brucellosis infection in a pastoralist community of Tanzania.

### 2.1.1 Study aims

The aims of this study were:

1. To identify risk factors for acute brucellosis infection in febrile patients from a pastoralist community presenting at a rural hospital in Tanzania

2. To compare the risk factors identified for this study population to risk factors previously described for acute human brucellosis studies in Tanzania and East Africa

## 2.2 Methods

### 2.2.1 Study site and population

This study was conducted at the Endulen Hospital in the Ngorongoro Conservation Area (NCA), Tanzania (see Figure 2.1). The NCA is an 8,292 km$^2$ multiple land use area designated for pastoralism, wildlife conservation and tourism (Government of Tanzania, 1996). The NCA has a human population of approximately 70,000 (NBS, 2013), largely comprised of semi-nomadic Maasai
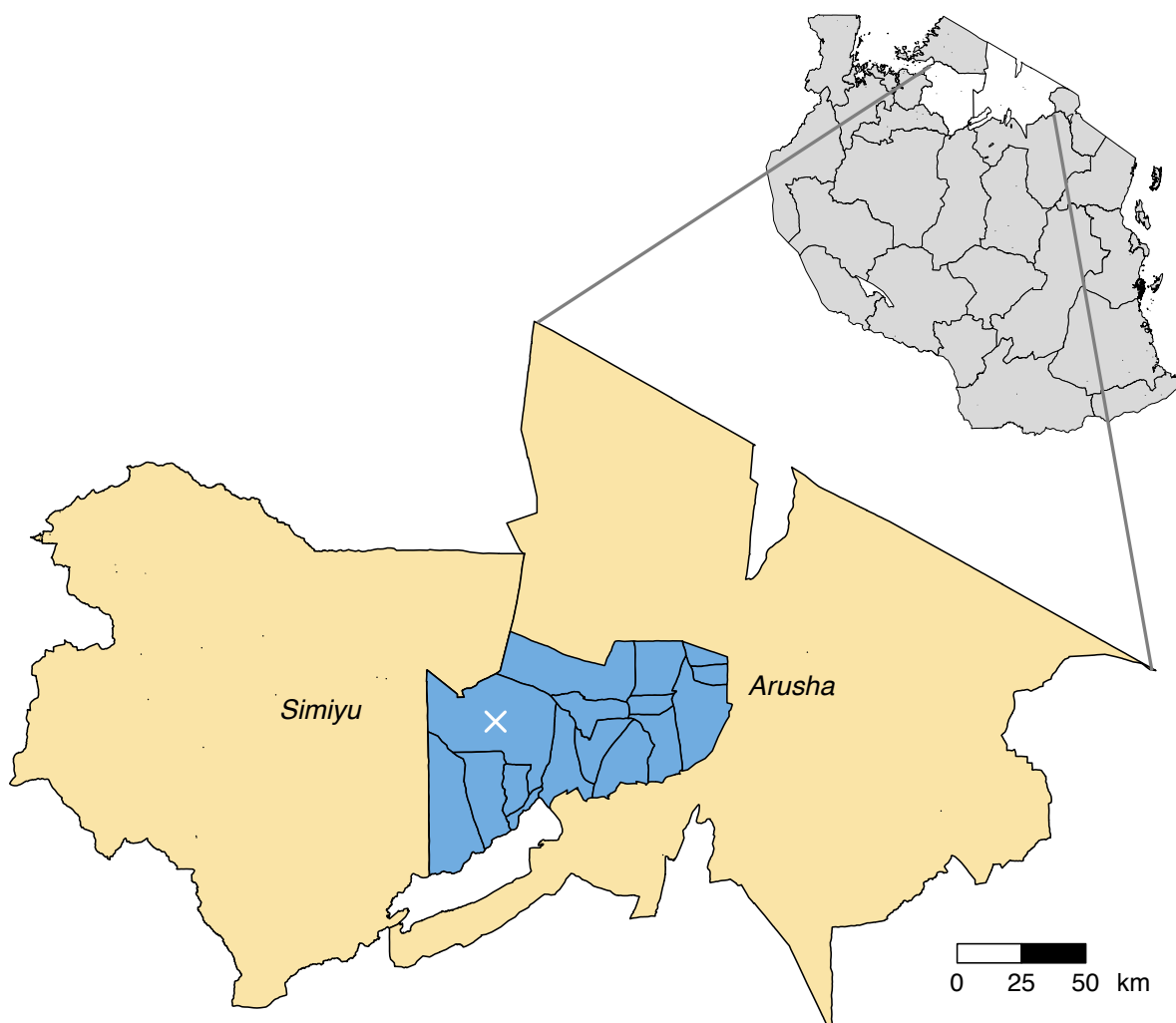


**Figure 2.1: The Endulen Hospital (white X) location within the Ngorongoro Conservation Area (blue shading) within Arusha Region and adjacent to Simiyu Region (beige shading), and the location of Arusha and Simiyu Regions within a regional map of Tanzania (grey shading)**, *polygon boundaries are shown for all villages within the NCA (blue shading). Shapefiles of administrative boundaries from the 2012 census were sourced from the Tanzania National Bureau of Statistics. Map reproduced from Bodenham et al., 2020, Creative Commons Attribution license: http://creativecommons.org/licenses/by/4.0/*

pastoralists (Allen et al., 2013). The main livestock species kept by the local Maasai community are cattle, sheep and goats (Government of Tanzania, 1996). The Endulen Hospital is a rural 110-bed hospital (Orsel et al., 2015), and the only hospital facility within the NCA. It is situated approximately 1900 meters above sea level, and the local area typically has a long rainy season from March to May and a short rainy period between October to December (NBS and ORC Macro, 2005).

## 2.2.2 Febrile hospital-based surveillance study

The risk factor analysis presented in this research chapter utilised data collected from a febrile hospital-based surveillance study (hereafter referred to as the febrile hospital study). The febrile hospital study was conducted by a larger research collaborative group to determine the prevalence of acute brucellosis in febrile hospital patients presenting at the Endulen Hospital. The methods described in Sections 2.2.3 – 2.2.6 refer to the methods employed in the larger febrile hospital study. These sections are described so as to understand the eligibility and enrolment protocol, blood sample collection and processing and questionnaire data collection performed that informed the subsequent risk factor analyses. RFB contributed to the larger febrile hospital study and was involved in the conceptualisation, design and implementation of the febrile hospital study, and designed the participant questionnaire. This chapter focuses on the risk factor analysis, all elements of which were performed by RFB. The methods detailed in Sections 2.2.7 onwards were performed specifically for the purpose of this research chapter and describe the risk factor analyses conducted here.

## 2.2.3 Febrile hospital study: Eligibility and enrolment

All patients attending the outpatient department (OPD) at Endulen Hospital with a tympanic temperature of ≥ 38.0 °C at the time of OPD presentation, or with a reported history of fever in the 72 hours prior to OPD presentation and aged two years or above were eligible for inclusion in the febrile hospital study. Eligible febrile hospital patients were enrolled into the febrile hospital study following

completion of written informed consent (see Appendix 1 for Participant Information and Consent Forms).

## 2.2.4 Febrile hospital study: Blood sample collection & processing

Following febrile hospital study enrolment, a blood sample was drawn by a clinical study team member for culture and acute-phase *Brucella* spp. serology testing. The blood sampling and sample bottle inoculation procedure was as follows: the participant's skin was cleaned around the selected blood draw site with isopropyl alcohol and povidone iodine. For febrile hospital participants weighing ≥25 kg, a 40 mL blood volume was collected at enrolment. Three culture bottles were inoculated with a target blood volume of 10 mL each: two BacT/ALERT (BioMérieux, Durham, NC, USA) aerobic culture bottles for automated culture; and a Castañeda (Ruiz Castañeda., 1961) bi-phasic media bottle. A plain vacutainer (BD, Franklin Lakes, NJ, USA) was filled with a target 10 mL of blood for serological testing and malaria testing was performed using SD BIOLINE Malaria Ag P.f/Pan rapid diagnostic test (Standard Diagnostics/Abbott, Abbott Park, IL, USA) or *CareStart* Malaria HRP2 (Pf) (ACCESS BIO, INC. Somerset, NJ, USA). Febrile hospital participants weighing <25 kg had a blood draw volume calculated based on weight. Sample bottle inoculation was the same as above, except that two paediatric BacT/ALERT bottles were inoculated for automated culture. Febrile hospital participants were approached four to six weeks after initial enrolment, for the collection of a target 10 mL blood volume which was inoculated into a plain vacutainer for convalescent-phase serology.

Inoculated culture bottles were transported at between 4-10 ℃ on the day of inoculation to the Kilimanjaro Clinical Research Institute (KCRI), Moshi, Tanzania. KCRI laboratory processing typically began the day after culture bottle inoculation and followed standardised protocols for identifying isolates (Crump et al., 2011b, 2011a).

Immediately following filling of the plain vacutainer, it was inverted 5 times and kept at ambient temperature for 45-60 minutes allowing clotting of the sample.

Following clotting, the vacutainer was spun at 1300-1500 g for 10 minutes in a centrifuge. Separated serum samples were pipetted into cryovials and stored at 4 °C at the Endulen Hospital before transfer at between 4-10 °C, with inoculated culture bottles, to KCRI. At KCRI, sera were stored at -80 °C. At the conclusion of the febrile hospital study participant enrolment, all sera were shipped on dry ice to the Animal and Plant Health Agency (APHA), Weybridge, UK. The APHA conducted serological testing by the serum agglutination test (SAT).

## 2.2.5 Definition: Acute brucellosis case

An acute brucellosis case was based on the CDC 2010 brucellosis case definition (CDC, 2010) and is defined as follows:

> *"A clinically compatible illness with:*
> *culture and identification of Brucella spp. (confirmed acute case)*
> *OR*
> *evidence of a four-fold or greater rise in Brucella antibody titre by SAT, between acute- and convalescent-phase sera obtained ≥2 weeks apart (confirmed acute case)*
> *OR*
> *a Brucella total antibody titre ≥160 by SAT in either acute- or convalescent-phase sera (probable acute case)"*

## 2.2.6 Febrile hospital study: Participant questionnaire

Following blood sampling of each febrile hospital participant, a study team member administered a structured, closed-ended questionnaire. Questionnaire topics included: demographic data; current and recent illness symptoms; reported history of brucellosis; dietary practices over the past 12 months; animal-related activities over the past 12 months; and pregnancy history, for adult female participants. The study team member read through each question in the preferred language of the febrile hospital participant (Maa or Swahili), completing the questionnaire with each febrile hospital participant individually, or in the presence of a parent or guardian. Where children were unable to respond, a parent or guardian assisted in completing the questionnaire on their behalf. Questionnaire data were collected using a paper-based OpenText

Teleform (OpenText, Waterloo, Ontario, Canada) format. Questionnaire administration was tested by the study team during the first three months of the febrile hospital study and was revised where necessary. All questionnaire data collected throughout the study were included in the final questionnaire dataset for analysis. Questionnaire data were digitised using the OpenText Teleform System, which generated an Access database (Microsoft Corporation, Redmond, WA, USA). The full febrile hospital study participant questionnaire can be found in Appendix 2.

## 2.2.7 Risk factor analysis: Questionnaire data cleaning

Analysing large datasets in order to investigate risk in epidemiological studies is common (Dohoo et al., 1997). The participant questionnaire for this study comprised a large number of questions that were considered candidate variables for risk factor analyses. Any candidate variable with more than 10% missing data was discounted and removed from the dataset. Candidate variables that were suspected of being poorly completed were also removed, such as any variable where interpretation of the question by the study population was reported as repeatedly challenging by the questionnaire administrator. Those candidate variables with multiple choice for the time period of reference, such as activities conducted over the last 30 days and over the last 12 months, were reduced to activities over the last 12 months. For identical questions repeated for individual livestock species (cattle, sheep and goats), responses were aggregated into a combined 'livestock' candidate variable. The outcome variable for the risk factor analyses was acute brucellosis status (case or non-case).

There is no single answer as to how many candidate variables are too many to include in a regression, however any regression analysis will be subject to overfitting and the validity of the model estimates compromised if too many candidate variables are included. One heuristic approach is that there must be 10 or more observations in the dataset per candidate variable included in the model (Dohoo et al., 2003a). Another suggestion is that to power a regression analysis, there should be a minimum of 50 observations, with a further 8 observations for each candidate variable included in the analysis and that the

number of observations should be increased further if the effect size is small (Green, 1991). Here, in order to minimise the risks of overfitting and improve the robustness of the risk factor analysis, three different data reduction methods were tested on the candidate variable dataset: multiple correspondence analysis (MCA); literature-informed logistic regression; and lasso regression. The outcomes of each method were considered in the decision for the final risk factor variables identified.

## 2.2.8 Risk factor analysis: Multiple correspondence analysis

MCA is a form of exploratory data analysis that can be used as a dimension reduction technique for categorical variables (Dohoo et al., 2003a). MCA is similar to principle components analysis, as it aims to detect a reduced set of orthogonal dimensions that maximise the explained variability in a large dataset (D'Enza and Greenacre, 2012). This technique therefore allows investigation into correlation between candidate variables and how imposed dimensions on the data are related to an outcome variable (Dohoo et al., 2003a). Usually, interpretation of MCA dimensions are restricted to the first two or three dimensions generated (Abdi and Valentin, 2007). A reduced dimension dataset selected by MCA can then be used to inform regression model construction.

Here, MCA was used to explore twenty-nine candidate variables, with acute brucellosis status (case or non-case) as the outcome variable. The only continuous variable, age in years, was discretised by creating age classes: 0-5 years; 6-12 years; etc. MCA does not support incomplete data, therefore the *missMDA* R package (Husson and Josse, 2019) was used to impute missing data. The *FactoMineR* R package (Husson et al., 2019) was used for MCA implementation. All data manipulation and analyses were performed in R software version 3.6.1 (R Core Team, 2019).

## 2.2.9 Risk factor analysis: Literature-informed logistic regression

In a literature-informed logistic regression, scientific literature identifying risk factors for human brucellosis infection in East Africa informed the selection of

candidate variables from the larger dataset. Epidemiologic opinion-informed candidate variables regarding livestock-human interactions and possible transmission routes were also selected for logistic regression. Univariable Bernoulli-distributed generalised linear models (GLM) were used to individually investigate each of the selected candidate variables' association with the outcome variable: acute brucellosis status (case or non-case). For the continuous variable age in years, a quadratic relationship with the outcome variable was suspected and therefore fitting a second order polynomial was investigated. Crude odds ratios (OR) and 95% confidence intervals (CI) were estimated.

Literature- or epidemiologic opinion-informed candidate risk factor variables were included in a multivariable Bernoulli-distributed GLM maximal model, with acute brucellosis status (case or non-case) as the outcome variable. For the age variable, inclusion of a quadratic polynomial was investigated again. Backward model selection was performed using likelihood ratio testing (LRT). Adjusted odds ratios (aOR) and 95% CI were calculated. The pseudo R-squared value is reported for maximal and final models.

A p-value ≤0.05 was considered statistically significant across all logistic regression analyses. All data manipulation and GLM analyses were performed in R software version 3.6.1 (R Core Team, 2019).

## 2.2.10 Risk factor analysis: Exploring candidate variable relationships

Any candidate risk factor variables identified as significantly associated with acute brucellosis status in univariable analysis and dropped from the final multivariable model during model selection were investigated in order to identify any collinearity with final model risk factor variables.

## 2.2.11 Risk factor analysis: Lasso regression

Lasso regression is a data shrinkage technique that penalises the estimated regression coefficients, these coefficients are constrained so the sum of the absolute value of the estimated coefficients is less than the constant $\lambda$ (Tibshirani, 1996). The result of applying this constraint means that some

candidate variables will be shrunk to zero and can therefore can be removed from the model (Harrell Jr., 2015). Cross-validation is used to determine $\lambda$.

A lasso regression was fitted to the candidate variables from the literature- and epidemiologic opinion-informed logistic regression maximal model. This was performed in order to verify whether a similar set of final candidate variables were selected using this penalised regression analysis, as compared to logistic regression backward model selection using LRT. A value of $\lambda$ one standard error (SE) greater than the minimised $\lambda$ was chosen. The binomial deviance loss function was specified as recommended for logistic regression type models. Cross-validation and lasso regression were performed using the *glmnet* R package (Friedman et al., 2019) in R software version 3.6.1 (R Core Team, 2019).

## 2.2.12 Research clearance and ethics

Implementation of the febrile hospital study was approved by the Tanzania Commission for Science and Technology, the Tanzania Wildlife Research Institute and the Ngorongoro Conservation Area Authority. The Kilimanjaro Christian Medical Centre Ethics Committee (698), the National Institute of Medical Research Tanzania (NIMR/HQ/R.8c/Vol.I/1140), the University of Otago Human Ethics Committee (H17/052), and the University of Glasgow College of Medical, Veterinary and Life Sciences Human Ethics Committee (200140149) gave ethical approval for this study. All research conducted was in accordance with the guidelines and regulations of the aforementioned organisations.

## 2.3 Results

### 2.3.1 Risk factor analysis data set

Between 15th August 2016 and 11th October 2017, 3,473 patients were screened at the Endulen Hospital. Of these patients, 435 (12.5%) were eligible for participation in the febrile hospital study. A total of 232 (53.3%) of 435 patients were enrolled and contributed data to analyses.

Overall, 230 (99.1%) of 232 febrile hospital participants had a blood sample collected for culture and/or serological testing. Of 230 febrile hospital participants, 130 (56.5%) were female and the median febrile hospital participant age was 27 years (range: 2 - 78 years). A total of 228 (99.1%) of 230 febrile hospital participants had at least one culture bottle inoculated. Bloodstream infections were detected in 14 (6.1%) of 228 febrile hospital participants. The different bloodstream infections identified are given in Table 2.1. Eight (3.5%) of 228 febrile hospital participants were *Brucella* spp. culture positive. One (0.4%) of 230 febrile hospital participants had a four-fold increase in antibody titre between acute- and convalescent-phase sampling by SAT. Therefore, 9 (3.9%) of 230 febrile hospital participants could be defined as confirmed acute brucellosis cases. A further 5 (2.2%) of 230 febrile hospital participants had a single SAT titre ≥160 and could be defined as probable acute brucellosis. In total, 14 (6.1%) of 230 febrile hospital participants met the definition for an acute brucellosis case. Thus, the outcome variable for risk

**Table 2.1: The number and proportion of febrile hospital participants (n=228) with evidence of a bloodstream infection by blood culture identified during the febrile hospital study**, *reproduced from Bodenham et al., 2020, Creative Commons Attribution license: http://creativecommons.org/licenses/by/4.0/*

| Microorganism | Total number (%) of febrile hospital participants with microorganism bloodstream infection |
|---|---|
| *Brucella* spp. | 8 (3.5) |
| *Enterococcus* spp. | 1 (0.4) |
| *Escherichia coli* | 1 (0.4) |
| *Salmonella enterica* | 1 (0.4) |
| *Salmonella enterica* serovar *Typhi* | 1 (0.4) |
| *Staphylococcus aureus* | 1 (0.4) |
| *Streptococcus pneumoniae* | 1 (0.4) |

factor analyses, acute brucellosis status, had 14 positive instances. Additionally, 6 (2.6%) of 230 febrile hospital participants were malaria rapid test positive. The febrile hospital study screening, eligibility, enrolment and brucellosis data collection steps are shown in Figure 2.2.
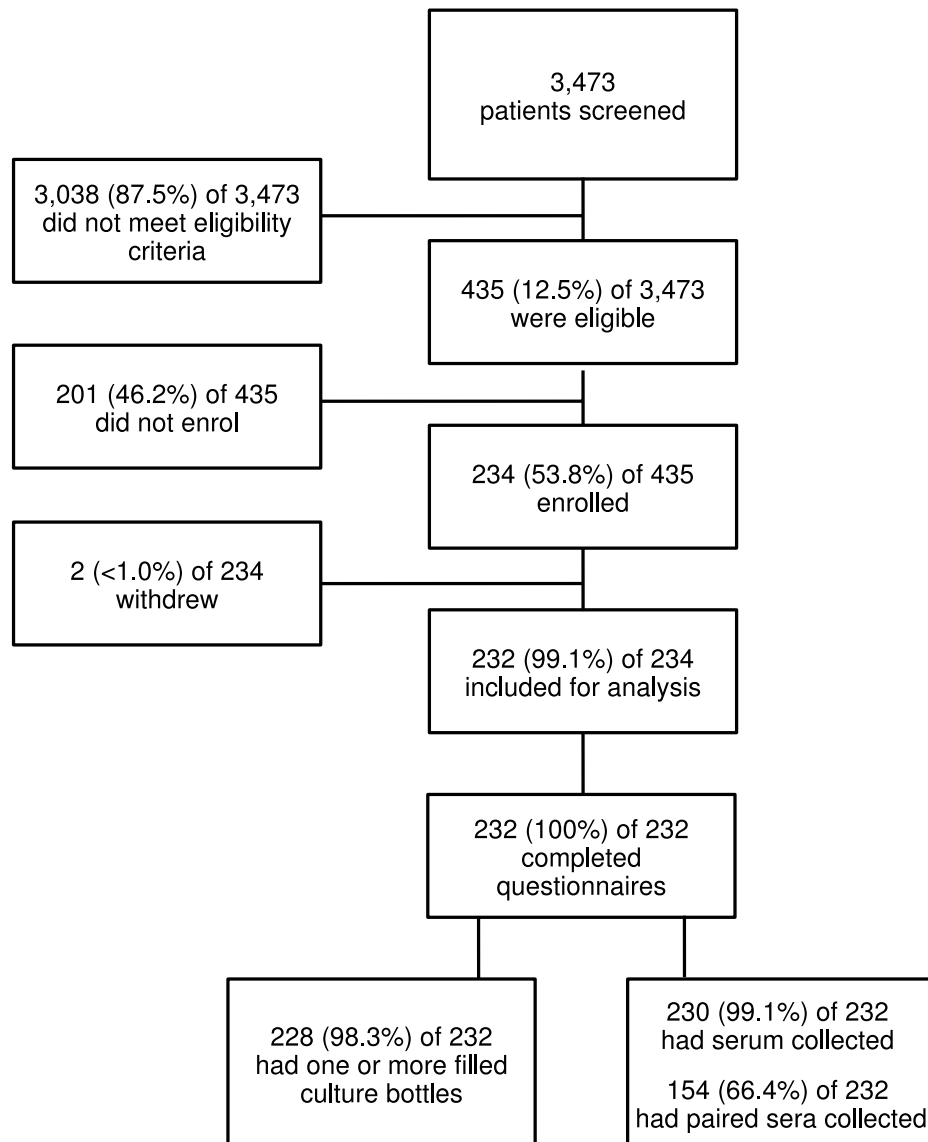


**Figure 2.2: Flowchart of febrile hospital study screening, eligibility, enrolment and brucellosis data collection figures**, *reproduced from Bodenham et al., 2020, Creative Commons Attribution license: http://creativecommons.org/licenses/by/4.0/*

All of the 230 febrile hospital participants had questionnaire data collected, 55 (23.9%) of 230 febrile hospital participants had questionnaire data collected during the initial questionnaire testing period. The raw data set contained 346 variables. Following the removal of variables with greater than 10% missing

values and any variables suspected to be poorly completed, as well as collapsing multiple choice variables, a total of 28 candidate variables remained.

## 2.3.2 Multiple correspondence analysis

The 28 candidate variables were included in an MCA. The outputs for the first three MCA dimensions were investigated. Dimension 1, 2 and 3 cumulatively explained 29.3% of the variance (see Table 2.2). Candidate variable factor levels (e.g. yes and no responses to risk factor questions) that contributed most to the construction of orthogonal dimensions 1, 2 and 3 are shown in Figure 2.3. Candidate variables identified in the first dimension explaining the most variance in the dataset were largely activities involving close contact with livestock, including: handled or had contact with any placental or birth products of livestock; assisted in the birthing of livestock; and herded any livestock. In the second dimension, the first candidate variables included demographic features such as marital status and age. The top ten contributing candidate variables to each of the first three MCA dimensions are described in Table 2.3.

**Table 2.2: Eigenvalues and proportion of variance explained by the first 5 dimensions of multiple correspondence analysis**

| Dimension | Eigenvalue | Variance (%) | Cumulative variance (%) |
|---|---|---|---|
| 1 | 0.177 | 14.354 | 14.354 |
| 2 | 0.101 | 8.158 | 22.512 |
| 3 | 0.083 | 6.750 | 29.262 |
| 4 | 0.070 | 5.669 | 34.931 |
| 5 | 0.064 | 5.188 | 40.119 |
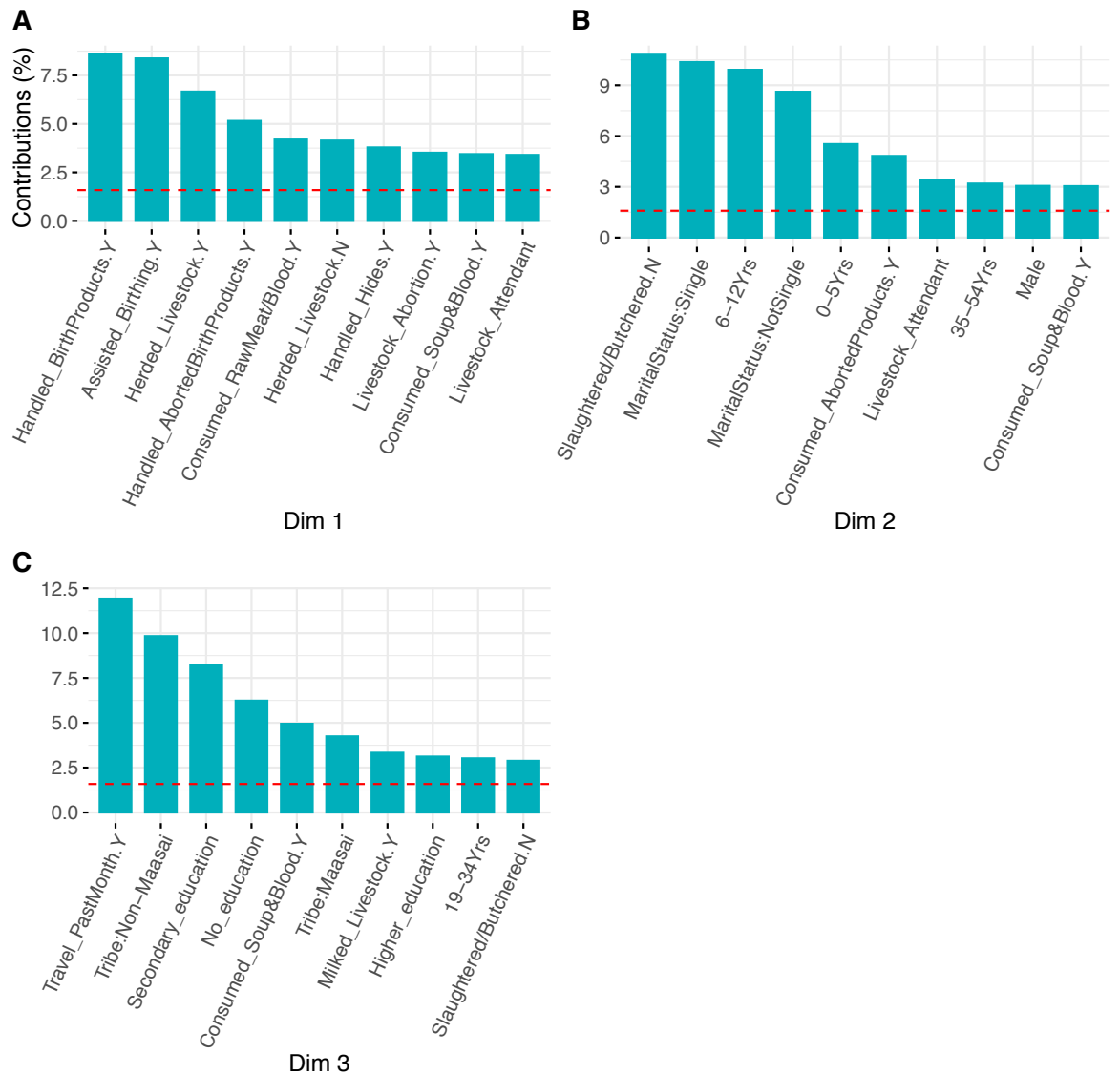
**Figure 2.3: The proportion of contribution of the top ten candidate variable factors to the construction of three multiple correspondence analysis orthogonal dimensions**, *panels A, B and C show the proportion of contribution of the top ten candidate variable factors for dimensions 1, 2 and 3, respectively, red dashed line shows the average expected value if all candidate variable factor contributions were equal, Y and N are Yes and No factor levels, respectively, candidate variables represent activity performed over the past twelve months where applicable or otherwise stated, animal-related candidate variables represent responses for cattle, sheep and goat species*

**Table 2.3: Risk factor candidate variables contributing to the first three dimensions of multiple correspondence analysis (MCA), including factor levels, candidate variable description and period of reference**, *N/A is not applicable, candidate variable names in parentheses are the names as used in MCA analyses and outputs*

| MCA candidate variable name | Factor levels | Description | Period of reference |
|---|---|---|---|
| Age | 0-5 yrs<br>6-12 yrs<br>13-18 yrs<br>19-34 yrs<br>35-54 yrs<br>55+ yrs | Age class of participant at the time of enrolment | N/A |
| Sex | Female<br>Male | Sex of participant | N/A |
| Marital status | Single<br>Not single | Marital status of the participant | N/A |
| Education | None<br>Primary_education<br>Secondary_education<br>High_school_education<br>Higher_education | Formal education level of the participant | N/A |
| Occupation | Livestock_Attendant<br>Other | The main occupation of the participant | N/A |
| Tribe | Maasai<br>Non-Maasai | The identified tribe of the participant | N/A |
| Travelled (Travel_PastMonth) | No<br>Yes | Has the participant travelled outside their home region? | Past month |
| Livestock abortions in herd or flock (Livestock_Abortion) | No<br>Yes | Have any cattle, sheep or goats from the participant's family herd or flock kept at the household aborted or delivered still-born offspring? | Past 12 months |
| Assisted with the birth of livestock (Assisted_Birthing) | No<br>Yes | Has the participant assisted with the birth of any cattle, sheep or goats? | Past 12 months |
| Milked livestock (Milked_Livestock) | No<br>Yes | Has the participant milked cattle, sheep or goats? | Past 12 months |
| Herded livestock (Herded_Livestock) | No<br>Yes | Has the participant herded cattle, sheep or goats? | Past 12 months |
| Slaughtered or butchered livestock (Slaughtered/ Butchered) | No<br>Yes | Has the participant slaughtered or butchered, or assisted in the slaughtering or butchering of cattle, sheep or goats? | Past 12 months |

| | | | | |
|---|---|---|---|---|
| Handled livestock hide (Handled_Hides) | No Yes | | Has the participant handled or prepared the hides of any cattle, sheep or goats? | Past 12 months |
| Handled livestock birth products (Handled_Birth Products) | No Yes | | Has the participant handled or had contact with any placental or both material of any cattle, sheep or goats? | Past 12 months |
| Handled livestock aborted materials (Handled_Aborted BirthProducts) | No Yes | | Has the participant handled or had contact with any aborted birth products from cattle, sheep or goats including: dead young or offspring; animal fluid; placenta; or blood? | Past 12 months |
| Consumed raw meat, offal or blood (Consumed_Raw Meat/Blood) | No Yes | | Has the participant consumed raw: meat; offal; or blood from cattle, sheep or goats? | Past 12 months |
| Consumed aborted livestock materials (Consumed_Aborted Products) | No Yes | | Has the participant consumed meat, offal or placenta from aborted cattle, sheep or goats? | Past 12 months |
| Consumed blood mixed with soup (Consumed_Soup& Blood) | No Yes | | Has the participant consumed soup with cattle, sheep or goats' blood? | Past 12 months |

### 2.3.3 Literature-informed logistic regression

A total of 18 literature- or epidemiologic opinion-informed candidate variables were selected from the larger 28 candidate variable dataset. Each of the 18 selected candidate variables are described in Table 2.4. These 18 candidate variables were then individually assessed using univariable GLM analysis, in order to investigate each of the selected candidate variables' association with brucellosis case status. A quadratic relationship for the candidate variable age was fitted to the univariable analysis, however it did not significantly improve model fit and so a linear relationship was assumed. Eight of the selected candidate variables caused inflation of the estimated standard errors most likely due to lack of data in acute brucellosis cases and were dropped from the analyses. For each of the 10 remaining candidate variables, the proportion of febrile hospital patient responses for each factor level are given in Table 2.5. The univariable analyses indicated a significant association between acute brucellosis cases and declining age in years (OR = 0.89, 95% CI = 0.83 - 0.95, p <0.001), male sex (OR = 3.50, 95% CI = 1.13 – 13.08, p = 0.039) and having herded any livestock in the past 12 months (OR = 10.85, 95% CI = 2.86 – 70.95, p < 0.01). Univariable analysis OR, 95% CI and p values are given for the 10 candidate variables in Table 2.5.

**Table 2.4: Literature- or epidemiologic opinion-informed candidate risk factor variables selected for inclusion in logistic regression analyses, including factor levels, candidate variable description and period of reference**, *N/A is not applicable, * represents candidate variables that were included in the final logistic regression analyses*

| Candidate variable | Factor levels | Description | Period of reference |
|---|---|---|---|
| Age* | Years (continuous) | Age of participant at the time of enrolment | N/A |
| Sex* | Female<br>Male | Sex of participant | N/A |
| Education | None<br>Primary<br>Secondary<br>High school<br>Higher | Formal education level of the participant | N/A |
| Occupation | Livestock attendant<br>Other | The main occupation of the participant | N/A |
| Livestock abortions in herd or flock* | No<br>Yes | Have any cattle, sheep or goats from the participant's family herd or flock kept at the household aborted or delivered still-born offspring? | Past 12 months |
| Assisted with the birth of livestock* | No<br>Yes | Has the participant assisted with the birth of any cattle, sheep or goats? | Past 12 months |
| Milked livestock* | No<br>Yes | Has the participant milked cattle, sheep or goats? | Past 12 months |
| Herded livestock* | No<br>Yes | Has the participant herded cattle, sheep or goats? | Past 12 months |
| Handled livestock waste* | No<br>Yes | Has the participant handled the manure of any cattle, sheep or goats including: during building construction; or cleaning animal pens? | Past 12 months |
| Slaughtered or butchered livestock* | No<br>Yes | Has the participant slaughtered or butchered, or assisted in the slaughtering or butchering of cattle, sheep or goats? | Past 12 months |
| Handled livestock carcass | No<br>Yes | Has the participant handled or had contact with the carcasses of any cattle, sheep or goats? | Past 12 months |
| Handled livestock hide | No<br>Yes | Has the participant handled or prepared the hides of any cattle, sheep or goats? | Past 12 months |
| Handled livestock aborted materials | No<br>Yes | Has the participant handled or had contact with any aborted birth products from cattle, sheep or goats including: dead young or offspring; animal fluid; placenta; or blood? | Past 12 months |
| Consumed raw dairy products* | No<br>Yes | Has the participant consumed raw dairy products including raw: milk, yoghurt, cheese, butter, | Past 12 months |

| Consumed raw meat, offal or blood* | No Yes | Has the participant consumed raw: meat; offal; or blood from cattle, sheep or goats? | Past 12 months |
|---|---|---|---|
| Consumed aborted livestock materials | No Yes | Has the participant consumed meat, offal or placenta from aborted cattle, sheep or goats? | Past 12 months |
| Consumed blood mixed with soup | No Yes | Has the participant consumed soup with cattle, sheep or goats' blood? | Past 12 months |
| Consumed blood mixed with milk | No Yes | Has the participant consumed blood mixed with milk from cattle, sheep or goats? | Past 12 months |

**Table 2.5: Univariable logistic regression results for ten literature- and epidemiologic opinion-informed candidate variables and the number and proportion of acute brucellosis cases at each candidate variable factor level**, *N is the total number of febrile hospital participants with data available, n is the number of acute brucellosis cases at each candidate variable factor level, % is the proportion of acute brucellosis cases, OR is crude odds ratios, CI is confidence intervals, p values reported to three decimal places, for Age in years median age and age range of acute brucellosis cases and total participants are reported*

| Candidate variable | | Acute brucellosis cases n/N (%) | Logistic regression OR (95% CI) | p value |
|---|---|---|---|---|
| Age in years median (range) | | 11 (7, 20)/28 (2,78) | 0.89 (0.83, 0.95) | <0.001 |
| Sex | Female | 4/130 (3.1) | Ref | |
| | Male | 10/100 (10.0) | 3.50 (1.13, 13.08) | 0.039 |
| Livestock abortions in herd or flock | No | 4/127 (3.1) | Ref | |
| | Yes | 8/88 (9.1) | 3.08 (0.94, 11.83) | 0.074 |
| Assisted with the birth of livestock | No | 11/170 (6.5) | Ref | |
| | Yes | 3/57 (5.3) | 0.80 (0.18, 2.69) | 0.743 |
| Milked livestock | No | 6/142 (4.2) | Ref | |
| | Yes | 8/83 (9.6) | 2.42 (0.81, 7.59) | 0.114 |
| Herded livestock | No | 2/134 (1.5) | Ref | |
| | Yes | 12/85 (14.1) | 10.85 (2.86, 70.95) | 0.002 |
| Handled livestock waste | No | 7/109 (6.4) | Ref | |
| | Yes | 7/119 (5.9) | 0.91 (0.30, 2.75) | 0.865 |
| Slaughtered or butchered livestock | No | 5/43 (11.6) | Ref | |
| | Yes | 9/184 (4.9) | 0.39 (0.13, 1.33) | 0.109 |
| Consumed raw dairy products | No | 7/167 (4.2) | Ref | |
| | Yes | 7/62 (11.3) | 2.91 (0.96, 8.86) | 0.055 |
| Consumed raw meat, offal or blood | No | 10/169 (5.9) | Ref | |
| | Yes | 4/60 (6.7) | 1.14 (0.30, 3.55) | 0.835 |

Multivariable analyses were not informed by univariable analysis outcomes. All 10 candidate variables were included in the maximal multivariable GLM so that backwards model selection could be performed. The model estimates for the 10 candidate variables included in the maximal model are given in Table 2.6. Stepwise model selection indicated that the most parsimonious model included: age of participant (LRT $\chi_2$= 18.17, df = 1, p < 0.001), with probability of acute brucellosis infection declining with age in years (aOR = 0.88, 95% CI = 0.81 - 0.94, p < 0.01); and participating in herding any livestock in the last 12 months (LRT $\chi_2$ = 11.71, df = 1, p < 0.001), with participating in herding increasing the probability of acute brucellosis infection (aOR = 10.16, 95% CI = 2.49 – 69.75, p < 0.01) (see Table 2.5). This final model had a pseudo R-squared value of 0.32 (see Table 2.6).

**Table 2.6: Multivariable logistic regression results for maximal and final models investigating association between ten literature- and epidemiologic opinion-informed candidate variables and febrile hospital participant acute brucellosis case status**, *aOR is adjusted odds ratios, CI is confidence intervals, LRT is likelihood ratio test, χ2 is Chi-squared statistic, df is degrees of freedom, AIC is Akaike Information Criterion*

| Candidate variable | | Maximal model | | | | Final model | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | aOR (95% CI) | aOR $p$ value | LRT $\chi_2$ | LRT $p$ value | aOR (95% CI) | aOR $p$ value | LRT $\chi_2$ | LRT $p$ value |
| Age in years | | 0.89 (0.76 – 0.97) | 0.047 | 7.46 | 0.006 | 0.88 (0.81, 0.94) | 0.002 | 18.17 | <0.001 |
| Sex | Female | Ref | | | | | | | |
| | Male | 20.73 (1.86 – 715.42) | 0.036 | 6.47 | 0.011 | | | | |
| | No | Ref | | | | | | | |
| Livestock abortions in herd or flock | Yes | 3.33 (0.58 – 26.69) | 0.204 | 1.77 | 0.184 | | | | |
| | No | Ref | | | | | | | |
| Assisted with the birth of livestock | Yes | 0.01 (0.00 – 0.18) | 0.007 | 11.71 | <0.001 | | | | |
| | No | Ref | | | | | | | |
| Milked livestock | Yes | 83.45 (5.17 – 4567.67) | 0.008 | 11.58 | <0.001 | | | | |
| | No | Ref | | | | Ref | | | |
| Herded livestock | Yes | 36.92 (2.49 – 1871.79) | 0.026 | 7.52 | 0.006 | 10.16 (2.49, 69.75) | 0.004 | 11.71 | <0.001 |
| | No | Ref | | | | | | | |
| Handled livestock waste | Yes | 0.05 (0.00 – 0.50) | 0.030 | 6.93 | 0.008 | | | | |
| | No | Ref | | | | | | | |
| Slaughtered or butchered livestock | Yes | 0.48 (0.03 – 7.07) | 0.588 | 0.29 | 0.587 | | | | |
| | No | Ref | | | | | | | |
| Consumed raw dairy products | Yes | 1.05 (0.10 – 8.71) | 0.966 | 0.00 | 0.966 | | | | |
| | No | Ref | | | | | | | |
| Consumed raw meat, offal or blood | Yes | 0.25 (0.02 – 2.49) | 0.264 | 1.35 | 0.245 | | | | |
| | | Null deviance = 90.66, df = 198 | | | | Null deviance = 104.09, df = 218 | | | |
| | | Residual deviance = 39.79, df = 188 | | | | Residual deviance = 71.82, df = 216 | | | |
| | | AIC = 61.79 | | | | AIC = 77.82 | | | |
| | | Pseudo R-squared = 0.62 | | | | Pseudo R-squared = 0.32 | | | |

## 2.3.4 Exploring the relationship between sex, age and herded livestock candidate variables

The candidate variables sex, age (years) and herded livestock were significantly associated with brucellosis case status in univariable analyses and the multivariable maximal model. Sex was not selected for inclusion in the final model. Relationships between sex and herded livestock, and sex and age (years) were evaluated for evidence of collinearity. A contingency table showing the raw descriptive data for febrile hospital participant sex and herded livestock candidate variables is given in Table 2.7.

**Table 2.7: Febrile hospital participants relationship between sex and herded livestock risk factor candidate variables**, *herded livestock is having herded cattle, sheep and/or goats in the past 12 months, N is the total number of febrile hospital participants with data available, n is the number of febrile hospital participants at each candidate variable factor level, CI is confidence intervals*

| | Herded livestock | | | |
|---|---|---|---|---|
| | | No | | Yes |
| Sex | n/N | % (95% CI) | n/N | % (95% CI) |
| Female | 96/134 | 71.6 (63.21 – 79.09) | 25/85 | 29.4 (20.02 – 40.29) |
| Male | 38/134 | 28.4 (20.91 – 36.79) | 60/85 | 70.6 (59.71 – 79.98) |

## 2.3.5 Lasso regression

All of the ten candidate variables that were included in the maximal multivariable logistic regression analysis were also fit to a lasso regression model. The model estimate for $\lambda$ one SE greater than minimised $\lambda$ was 0.041. Eight of the ten candidate variables shrank to zero. The two covariables selected by lasso regression were age and herded livestock, see Table 2.8 for candidate variable coefficient estimates.

**Table 2.8: Lasso regression coefficient estimates (Coef.) for non-zero candidate risk factor variables**

| Candidate variable | | Lasso regression Coef. |
|---|---|---|
| Age in years | | -0.04 |
| Herded livestock | No | Ref |
| | Yes | 0.98 |

## 2.4 Discussion

This study is the first risk factor analysis of acute brucellosis infection for a pastoralist community in Tanzania, and one of few studies reported for East Africa. Febrile hospital participant questionnaire data were considered as candidate variables for risk factor analyses. The candidate variable dataset was large, so in order to avoid overfitting of the data and increase the robustness of a risk factor analysis, three different data reduction techniques were applied to the data: MCA; literature-informed logistic regression; and lasso regression. The final risk factors identified for acute brucellosis infection in febrile hospital participants were: having herded livestock; and decreasing age in years.

An MCA was applied to the candidate variable dataset The MCA grouped candidate variables according to the proportion of variance explained by the construction of each orthogonal dimension. Broadly, the first dimension included candidate variables involving livestock contacts and the second dimension included candidate variables describing demographic characteristics. However, dataset simplification resulting from dimension reduction was not sufficient to mitigate the reduced interpretability caused by grouping of the candidate variables. Therefore, the outputs of the MCA analysis were not considered as an effective candidate variable reduction technique for this dataset and did not further inform risk factor analyses in this study.

In the literature- and epidemiologic opinion-informed multivariable logistic regression, two risk factors for acute brucellosis in febrile hospital participants were identified in the final model. These risk factors were: having herded cattle, sheep and or goats in the past 12 months; and decreasing age of the participant. Sex was a significant candidate variable in univariable analysis, however was not included in the final multivariable model. Inspection of the relationship between sex and having herding livestock indicated collinearity between these candidate variables, with males more likely to herd livestock. Additionally, collinearity was identified between sex and age candidate variables, with males more likely to be of younger age.

The logistic regression maximal model included 10 candidate variables. It was therefore possible that the backwards model selection used to determine the

final model risk factor variables was influenced by overfitting of the data. Lasso regression data reduction was performed to further investigate the same 10 candidate variables. Lasso regression selected the same two candidate variables as the logistic regression model. This suggests that the two identified risk factors for acute brucellosis do have a true effect on acute brucellosis status and are not an artefact of overfitting.

The pseudo R-squared value for the final logistic regression model indicated that 0.32 of variation was explained by the model. Therefore, the final model does not capture all of the variation in risk of acute brucellosis in this population. It is likely that there are additional risk factors for acute brucellosis in this setting that have not yet been identified. Increasing the sample size may lead to the detection of additional risk factors for acute brucellosis. Achieving a larger acute brucellosis case sample size in this population however, would be a significant logistical challenge.

The risk factors identified in this study, being a young person and involved in herding, do not align explicitly with other risk factor studies conducted in Tanzania or East Africa. In the only other study of risk factors for acute brucellosis in Tanzania, Cash-Goldwasser *et al*. conducted a study of febrile patients from a largely urban-based community in Kilimanjaro Region. Brucellosis prevalence by BMAT was reported as 8.9% (n = 562) and risk factors for acute brucellosis included: assisting in small ruminant births; contact with cattle; and consumption of boiled dairy products which was protective against acute brucellosis (Cash-Goldwasser et al., 2018). In the study reported in this thesis, a brucellosis prevalence of 6.1% (n = 230) was detected using a combination of culture and SAT. The differences in study design between the two studies, including: diagnostics used; and study location, including size of study hospital, may in part account for differences in brucellosis prevalence and risk factors identified. The difference in risk factors identified could also be explained by differences in *Brucella* spp. exposure risk between the largely rural versus largely urban study populations. There may be important differences in *Brucella* spp. transmission pathways in different settings. Here, the study area was rural, and the community were almost exclusively pastoralists. Behavioural practices, such as livelihood activities, and their linked *Brucella* spp.

transmission risks for this study population are very different to the livelihood activities of a study population from an urban area. Additionally, the median age of infection for acute brucellosis was 11 years and for Cash-Goldwasser *et al.* it was 31 years. In nomadic communities such as that of the Maasai of the NCA, it has been suggested that members of the community are exposed to *Brucella* spp. from a young age, and as a result adults do not manifest acute disease but may be suffering from chronic infection (Corbel, 2006). Therefore, perhaps the difference in identified risk factors between these two studies could be explained by a more common, constant *Brucella* spp. exposure risk in endemic rural settings as compared to urban settings. The prevalence detected in these febrile patient studies was slightly higher in the study reported by Cash-Goldwasser *et al.* and the urban referral hospital, as compared to the rural hospital in the study reported in this thesis. A higher human brucellosis burden in rural, pastoral settings as compared to other livestock-keeping settings has been reported previously in Tanzania (Shirima et al., 2010). The difference in prevalence estimates reported for the Cash-Goldwasser *et al.* study and the study reported here could have been influenced by differing hospital catchment populations, as well as the health seeking behaviour of the febrile population.

Another explanation for the difference in identified acute brucellosis risk factors may be that not all acute brucellosis cases present to a healthcare provider, and that those that do reach a healthcare facility in rural versus urban study areas are very different. In rural areas there are many barriers to accessing healthcare facilities, some include: distance to healthcare facility; lack of funds for treatment; and the inability to lose the time required to visit a healthcare facility (Maudlin et al., 2009). Thus, it may be that more severely symptomatic individuals are prioritised for visiting a healthcare provider, meaning that an even smaller proportion of acutely infected individuals make it to a healthcare facility in rural settings than urban settings. Barriers to healthcare therefore can impact on the risk factors detected in different study locations and populations.

A study of a largely pastoralist community of Kenya investigating brucellosis in febrile patients similarly found contact with multiple animal species to be a significant risk factor for acute brucellosis infection, as well as reporting herding as an occupation (Njeru et al., 2016a). However, reporting herding as an

occupation is arguably not the same classification as a participant reporting they have been involved in herding over the past 12 months. In the present study, the age range of acute brucellosis cases was 7 to 20 years old and likely describes a different demographic of individuals to those who might report their occupation as herding. Indeed the brucellosis positive patients in the Kenya-based study ranged from 23 to 46 years old (Njeru et al., 2016a).

When comparing the risk factors identified in this study to those reported in other studies, it is important to consider the stage of brucellosis infection that has been measured. For example, in a cross-sectional study of a pastoralist community in Kenya, increasing age by decade was significantly associated with evidence of *Brucella* spp. exposure (Osoro et al., 2015). A Ugandan study also found that female patients were significantly more likely than males to be exposed to *Brucella* spp. and that female patients were significantly older than male patients exposed to *Brucella* spp. (Makita et al., 2011b). These findings are the converse of the present study. The diagnostic tests used in Osoro *et al*. and Makita *et al*. detected any antibody response to *Brucella* spp. exposure, which will have included (and not differentiated between) active brucellosis cases and historic exposure to *Brucella* spp. In endemic areas, it is likely that adult members of the community will have persistent *Brucella* spp. antibodies due to repeat exposures and test serologically positive (Al Dahouk and Nöckler, 2011). Therefore, *Brucella* spp. exposure may increase with age. In this study, the case definition was specific to acute infections, and therefore would have resulted in underestimation of more advanced stages of brucellosis infection or historic exposure.

Surprisingly, consumption of raw dairy products was not identified as a risk factor in the present study. Previously, in East Africa raw milk consumption has been frequently identified as a risk factor for acute brucellosis (Asiimwe et al., 2015; Kiambi, 2012; Njeru et al., 2016a), or consumption of boiled dairy products has been reported as protective against infection (Cash-Goldwasser et al., 2018). However, it is possible that responses to the raw milk consumption practices question were subject to a type of questionnaire bias referred to as 'faking good', whereby the participant alters their response to a response they perceive to be preferred by the investigator (Choi and Pak, 2005). Brucellosis is

considered to be endemic in pastoralist communities (McDermott et al., 2013) and when visiting a healthcare facility patients may be commonly tested for brucellosis. There have also been a number of brucellosis sensitisation activities conducted in this study community and febrile hospital participants were aware that they were participating in a study on human brucellosis. Therefore, it is possible that only 50.0% of acute brucellosis cases reported raw dairy product consumption because the questionnaire was subject to faking good bias. Raw dairy consumption may be a confounder variable and with a larger sample size, or further investigation into young herding individuals in the community, it could prove to be a risk behaviour. Alternatively, it is also possible that in this community, where only 6.1% of the total study population reported consuming raw dairy products, there is a behavioural shift towards milk boiling.

Following consideration of the literature on risk factors for acute brucellosis infection within East Africa, it would appear that broad risk factor categories are similar across various settings, such as general contact with livestock species. However, details of the precise livestock-related activities or the demographic at highest risk appear to vary by study. Focusing on this study community, it is common that younger individuals, particularly boys, are given responsibility for herding cattle, sheep and goats (Mangesho et al., 2017). There are many activities conducted whilst herding that could increase the probability of transmission of *Brucella* spp. including: contact with livestock deaths; butchering livestock and ingesting raw organs or undercooked meat and blood; assisting livestock births; contact with new-born livestock; and consuming raw milk directly from livestock (*personal communication with community members*). The probability of transmission of *Brucella* spp. whilst herding is further increased due to the unavailability of basic hygiene measures, such as soap and water for handwashing.

Finally, in thinking about the larger febrile hospital study results (as opposed to the aspects that directly contributed to this research chapter only), seven distinct bloodstream infections were identified in 6.1% of febrile hospital participants sampled for culture. *Brucella* spp. infections were the most frequently detected bloodstream infection, identified in 3.5% of those febrile participants sampled (Bodenham et al., 2020). Other human bloodstream

infection studies within Africa report *Brucella* spp. as a rare cause of infection as compared to other bloodstream infections, such as *Salmonella enterica* or *Streptococcus pneumoniae* (Marchello et al., 2020; Reddy et al., 2010). Malaria, as diagnosed by rapid diagnostic testing, was also a relatively infrequent cause of febrile illness. Malaria was identified in only 2.6% of febrile participants, whereas a total of 6.1% of febrile participants were identified as brucellosis cases (Bodenham et al., 2020). Diagnostic testing for leptospirosis (*Leptospira* spp.) has also detected a comparable number of *Leptospira* spp. exposures to brucellosis cases for the febrile hospital study participants (Maze MJ & Halliday JEB, unpublished data). These febrile hospital study results highlight the importance of human brucellosis in this rural pastoralist community as compared to other causes of febrile illness.

A limitation of this study was that questionnaire data collection can be susceptible to a variety of biases. For example, data collection may have been subject to recall bias, whereby respondents' accuracy in recalling past events varies (Choi and Pak, 2005). Another example is that of response fatigue. The questionnaire administration took approximately 40 minutes, due to response fatigue during that time respondents may have given inaccurate or repetitive responses (Choi and Pak, 2005). Restricting the length of the questionnaire is an obvious way to reduce response fatigue. Additionally, randomisation of question ordering may reduce systematic bias for questions that were repeatedly answered poorly due to response fatigue, potentially because they were positioned towards the end of the questionnaire or grouped together and repetitive. An alternative approach to reducing questionnaire bias is through the implementation of other data collection techniques in the study population, such as focus group discussions or key-informant interviews in an attempt to verify questionnaire data collection. It is also important to consider that in suspected high brucellosis-risk communities, effectiveness of traditional risk factor analyses may be limited due to a large proportion of the community being involved in the broad risk factors for brucellosis transmission such as direct contact with animals. It is also important to highlight that the findings of this study must be interpreted in line with the study design used, particularly with respect to the denominator population. The risk factors for acute brucellosis cases identified in this study are those applicable to febrile hospital

participants. As the non-brucellosis case febrile hospital participants may have been involved in risk factors that overlapped brucellosis and other febrile illnesses, this may have influenced the ability of the study to identify risk factors for acute brucellosis. This could have been reduced by choosing a random community control group, although this approach would have been much more resource-intensive. Another consequence of study design and the sampled population was selection bias introduced at the different steps of study screening and enrolment, for example only 53.8% of eligible patients were enrolled into the study. This selection bias may have been for a number of reasons including that eligible patients may have refused participation because they were focused on receiving care for their current illness, or because the family decision maker was not present. Due to the high proportion of eligible febrile patients not enrolled in the study, it is possible that the prevalence of acute brucellosis in febrile patients was biased. One way to reduce selection bias could be to combine hospital surveillance with active community-based surveillance.

Further research might explore the risk factors for acute brucellosis identified in this study by investigating the herding-specific activities that are increasing *Brucella* spp. transmission for young herders. Sensitisation to the risks of brucellosis transmission in this high-risk demographic group would also be a logical next step. These risk factor data coupled with further investigation would be invaluable in informing brucellosis prevention and control interventions in the NCA.

## 2.5 Conclusion

The need for effective, achievable brucellosis control is great in endemic pastoralist communities (Racloz et al., 2013). Understanding setting-specific risk factors for brucellosis infection is a vital tool in the formation of an efficient, evidence-based disease prevention and control strategy. The investigation of risk factors associated with acute disease is particularly important, as this allows identification of temporally relevant risk practices related to active infection. This is one of the first studies in East Africa to identify risk factors associated with acute brucellosis infection. Risk factors included herding livestock and

decreasing age in years. It is recommended that these data are used to inform further research that investigates the herding-specific risk activities that put young herders at high risk of infection. The consideration of these risk factors in the development of brucellosis prevention and control interventions has the potential to make a substantial impact on human brucellosis burden in this pastoralist community, as well as in similar communities in Tanzania and wider SSA.

# Chapter 3 Human brucellosis active surveillance: Screening household members of febrile hospital patients

### 3.1 Introduction

Zoonotic disease burden is disproportionately high among impoverished pastoralist communities (WHO et al., 2006). These communities are believed to be particularly vulnerable to the impacts of endemic zoonoses due to reasons that include living in close contact with livestock, reduced availability of healthcare facilities in rural areas, and inability to afford both treatment and the time lost by visiting a healthcare provider (Maudlin et al., 2009). Consequently, the burden of zoonotic diseases in these endemic areas is often underestimated (Maudlin et al., 2009).

Human disease surveillance data can play an important role in understanding the true burden of endemic diseases, especially in impoverished communities (Halliday et al., 2012). Disease surveillance is also fundamental in informing effective disease control measures, and has been referred to as "the foundation for the control of infectious diseases" (Berkelman et al., 1994). The World Health Organization (WHO) defines disease surveillance as "the ongoing systematic collection, analysis and interpretation of health data needed for planning, implementation and evaluation of public health practice" (WHO, 2006). There are two main mechanisms of disease surveillance: passive and active. Passive surveillance is the routine collection of disease data by healthcare facilities and laboratories that is reported to the appropriate health authority (WHO, 2020). In contrast, active surveillance involves active searching for cases of infectious disease in the community, such as regular phone calls to clinicians and visits to healthcare centres and laboratories (Kramer et al., 2010).

Passive disease surveillance in the form of routine clinical reporting is advantageous in its potential to generate longitudinal data, in its role in early detection of disease outbreaks, and relative low cost (Hadorn et al., 2008; Robinson, 2003). However, this form of surveillance suffers from a lack of control over the quality and often the detail of data collected (Hattendorf et al., 2017). Passive surveillance in the form of hospital-based surveillance may

underestimate the burden of disease. There are multiple reasons for this, including: social, economic and geographical barriers to accessing healthcare; clinical and laboratory diagnostic misdiagnosis of patients; and high levels of under-reporting (Dean et al., 2012b; WHO et al., 2006). In general, under-reporting is thought to be due to various factors that can be largely categorised as unwillingness (e.g. due to: lack of compensation; or negative consequences of reporting, such as slaughter or trade bans) or the inability (e.g. due to: lack of diagnostic testing; or lack of awareness by patient, clinician or livestock-keeper; lack of communication between stakeholders) to report on zoonotic disease (Halliday et al., 2012). Regarding brucellosis surveillance, a study in Greece investigating passive reporting of hospital records to the public health department found that 38.0% of notifiable infectious diseases were not reported, including 26.0% of brucellosis cases not reported (Jelastopulu et al., 2010). A study in Moshi, Tanzania analysed 528 hospital clinical diagnoses of febrile patients (Crump et al., 2013). No clinical diagnosis of brucellosis was recorded, however study diagnostic testing showed that 3.5% of febrile patients had acute brucellosis (Crump et al., 2013). These findings indicate that passive surveillance reliant on clinical diagnosis alone can result in under-reporting of brucellosis. Conversely, hospital-based passive surveillance data can also lead to overestimation of disease burden. For example, the routine use of brucellosis point-of-care diagnostic tests with low specificity, combined with the absence of confirmatory testing, can result in overdiagnosis of brucellosis (de Glanville et al., 2017). Implementation of suboptimal diagnostic testing procedures may often be performed in resource-limited settings and will ultimately misrepresent the burden of disease. Another issue regarding the ability of passive surveillance to improve our understanding of the burden of brucellosis is disease prevalence. In endemic areas, such as in the agro-pastoral and pastoral areas of Tanzania, cross-sectional surveys estimate human brucellosis seroprevalence to range from 0.0% to 8.3% (Assenga et al., 2015; Shirima et al., 2010; Shirima and Kunda, 2016). If the true seroprevalence in these endemic areas lies within this range, then low brucellosis prevalence coupled with passive surveillance limitations such as under-reporting, make understanding the burden of brucellosis and informing disease prevention and control via passive surveillance a challenge.

Data generated through active surveillance can be more accurate compared with passive surveillance (Mphande, 2016), temporally, spatially and demographically. Active surveillance data combined with passive surveillance can provide more representative data and an effective brucellosis surveillance strategy should combine passive data acquisition with active surveillance wherever possible (Robinson, 2003). However, active surveillance is generally expensive to implement, especially in the form of randomised field surveys. Randomised field surveys may also fail to effectively represent diseases that are spatially grouped (Hattendorf et al., 2017). This form of active surveillance may be especially problematic for brucellosis, which is considered to be spatially grouped in risk populations (WHO et al., 2006).

Adapted active surveillance strategies have been applied to various infectious diseases in order to gain a better understanding of disease burden in hard to reach populations, that potentially have a grouped distribution. One example is contact tracing, this technique is used in the identification of potential rabies virus exposures. A single report of an animal bite victim at a healthcare facility is investigated and an interview with the victim or family is conducted in order to identify the source of exposure and any additional bite victims (also referred to as contacts) (Hampson et al., 2008). This process is then repeated for each identified contact (Hampson et al., 2008). A similar technique, referred to as active case finding, has been implemented among tuberculosis (*Mycobacterium tuberculosis*) cases in many countries globally and is reported to improve case detection rates (Golub et al., 2005). An example of the implementation of active case finding for tuberculosis is through screening of household members of tuberculosis patients (Zachariah et al., 2003).

The household members of hospital patients with brucellosis are likely to share many risk factors for the disease such as meat and dairy consumption practices, therefore these individuals are at high risk of exposure (Tabak et al., 2008). Consequently, active case finding in the form of brucellosis screening household members of brucellosis patients in endemic settings has been advocated (Moreno, 2014). Active case finding, as compared to randomised field survey techniques, may prove a more effective form of active surveillance for the detection of *Brucella* spp. exposures in settings where there are close contacts

with livestock species and/or consumption of raw animal products. In Eastern Europe, the Middle East, and Latin America, screening of household members of brucellosis patients for exposure to *Brucella* spp. has been implemented. These studies report that the screening of household members of brucellosis patients leads to increased detection of *Brucella* spp. exposures, including detection of acute brucellosis cases (Almuneef et al., 2004; Alsubaie et al., 2005; Ismayilova et al., 2013; Mendoza-Nunez et al., 2008; Sanodze et al., 2015; Sofian et al., 2013; Tabak et al., 2008).

Similar examples of household member screening for brucellosis in SSA are lacking, with brucellosis surveillance of any form seldom implemented in pastoral areas (Njeru et al., 2016b). Yet, studies investigating risk factors for human brucellosis in East Africa have identified a positive association between family history of brucellosis and exposure to *Brucella* spp. in febrile hospital patients (Asiimwe et al., 2015; John et al., 2010; Migisha et al., 2018), and suggest the importance of household member brucellosis screening as a tool in the control of brucellosis (Asiimwe et al., 2015; Migisha et al., 2018). In Tanzania, a study at a referral hospital in Moshi, estimated that only 4% of people with febrile illness attended hospital, study participants instead reported a preference for self-management of fever (Panzner et al., 2016). Active surveillance in Tanzania may therefore be especially effective in detecting *Brucella* spp. exposures, including acute brucellosis cases, and in generating a more accurate estimate for the burden of brucellosis.

There are no evident examples in the scientific literature for Africa of studies implementing brucellosis active surveillance through the screening of household members of brucellosis patients. In this study, the ability of an active case finding approach to detect brucellosis in household members of febrile hospital patients will be evaluated.

### 3.1.1 Study aims

The overall aim of this study was to investigate whether screening of household members of febrile hospital patients with acute brucellosis could detect additional acute brucellosis cases or *Brucella* spp. exposures, and to evaluate evidence of grouping in the distribution of *Brucella* spp. exposure status of household members.

The five study aims were:

1. To determine if new acute brucellosis cases could be identified amongst the household members of febrile hospital participants with acute brucellosis

2. To estimate the prevalence of *Brucella* spp. exposure amongst the household members of febrile hospital participants

3. To evaluate evidence of association between the *Brucella* spp. exposure status of household members and febrile hospital participants

4. To compare the age and sex distributions of febrile hospital study participants (as described in Chapter 2) and household member study participants

5. To compare the age and sex distributions of RBT-defined *Brucella* spp. exposed febrile hospital study participants (as described in Chapter 2) and household member study participants

## 3.2 Methods

### 3.2.1 Study area

This prospective study was conducted in the Ngorongoro Conservation Area, Tanzania. See Chapter 2 Section 2.2.1 for description of the study site and population.

### 3.2.2 Febrile hospital study

The febrile hospital study at the Endulen Hospital enrolled eligible and consenting patients presenting to the outpatient department (OPD) between 15th August 2016 and 11th October 2017 (see Chapter 2 Section 2.2.3 for further details of the febrile hospital study eligibility and enrolment protocol). All febrile hospital participants enrolled into the febrile hospital study between 5th April and 11th October 2017 were eligible for household member sampling. During this period, the febrile hospital participants were informed at the time of their enrolment that they may be approached for additional data collection and household follow-up visits in order to identify acute brucellosis cases or evidence of *Brucella* spp. exposures in their household.

### 3.2.3 Definitions: Acute brucellosis case and *Brucella* spp. exposure

- An **acute brucellosis case** is defined as:

  *"A clinically compatible illness with: culture and identification of Brucella spp. (confirmed acute case); or evidence of a four-fold or greater rise in Brucella antibody titre by serum agglutination test (SAT), between acute- and convalescent-phase sera obtained ≥2 weeks apart (confirmed acute case); or a Brucella total antibody titre ≥160 by SAT (probable acute case)"* (CDC, 2010)

- ***Brucella* spp. exposure** is defined as:

  An individual classified as an acute brucellosis case

  AND/OR

  seropositive by the Rose Bengal plate test (RBT)

### 3.2.4 Febrile hospital participant sampling and RBT testing

All febrile hospital participants had a blood sample drawn for culture and acute-phase serology at the time of hospital presentation and four to six weeks following hospital presentation were approached for a second blood sample for convalescent-phase serology (see Chapter 2 Section 2.2.4 for full details of the febrile hospital study blood sample collection and processing protocol).

Febrile hospital participant serum was tested by the Rose Bengal plate test (RBT) at the Endulen Hospital within a week following febrile hospital participant enrolment. RBT testing was performed following standard protocols (Corbel, 2006; Díaz et al., 2011) as follows: serum, RBT antigen, positive and negative controls (Animal and Plant Health Agency (APHA), Weybridge, UK) were brought to ambient temperature. Serum was inverted several times and RBT antigen and controls were shaken to ensure homogenisation of the suspension. A volume of 25 μL serum was pipetted onto a glossy white ceramic tile, and an equal volume of antigen pipetted next to the serum sample. Serum was mixed thoroughly with the antigen using a clean toothpick, producing an approximately 2 cm oval-shaped suspension per serum sample. For each tile, 25 μL positive and negative control were included, and an equal volume of antigen pipetted next to each control. Controls and antigen were also mixed using a clean toothpick. The tile was then rotated and tilted by hand for 4 minutes. After four minutes, the tile was read in a well-lit environment. Any visible clumping of the antigen was identified as a positive result (see Fig. 3.1).
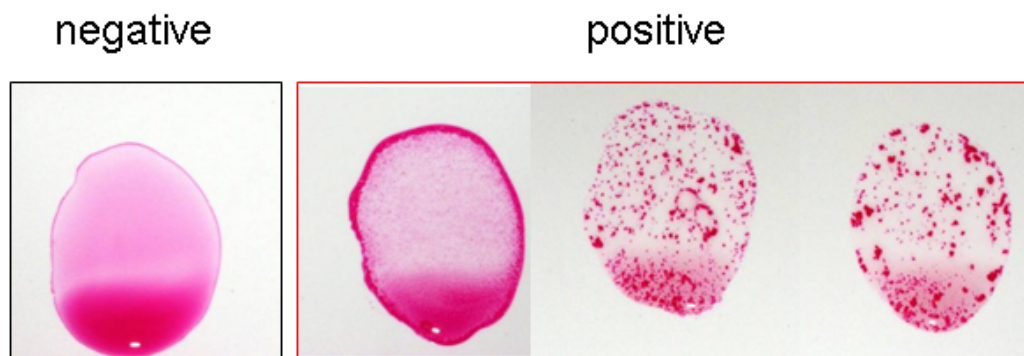


**Figure 3.1: Examples of negative and positive agglutination using the Rose Bengal plate test (RBT)**, *reproduced from Diaz et al., 2011, Creative Commons Attribution license: https://creativecommons.org/licenses/by/4.0/*

### 3.2.5 Definitions: Brucellosis positive and brucellosis negative febrile hospital participants

Brucellosis Positive Hospital participants (hereafter referred to as BPH) were defined as:

- **BPH:**

  Febrile hospital participants explicitly diagnosed as:

  an acute brucellosis case

  OR

  were classified as *Brucella* spp. exposures

Brucellosis Negative Hospital participants (hereafter referred to as BNH) were defined as:

- **BNH:**

  Febrile hospital participants that did not meet the criteria for:

  an acute brucellosis case

  AND

  were not classified as *Brucella* spp. exposures

### 3.2.6 Household classification

A list of febrile hospital participants was generated 1 to 4 days following hospital enrolment to determine the order of approach of febrile hospital participants for household follow-up visits. At the time of household follow-up, febrile hospital participant RBT was the only blood test available to guide identification of BPHs, as culture and SAT results were only available months after initial data collection. The approach list was semi-randomised in that prioritisation was given to BPHs. The approach list was updated every 1-4 days to include newly enrolled febrile hospital participants. At the conclusion of the study, SAT and culture results were used to retrospectively inform classification of BPHs and BNHs and their households. See Figure 3.2 for further description of household classification beginning with a febrile patient's first presentation at the Endulen Hospital OPD.

### 3.2.7 Definitions: BPH and BNH households

The Brucellosis Positive Hospital participant Household (hereafter referred to as BPHH) was defined as:

- **BPHH:**

  All individuals in a compound (a group of houses and animal pens) where members of multiple houses may share ownership of the same livestock as the BPH

The Brucellosis Negative Hospital participant Household (hereafter referred to as BNHH) was defined as:

- **BNHH:**

  All individuals in a compound (a group of houses and animal pens) where members of multiple houses may share ownership of the same livestock as the BNH

### 3.2.8 Household selection

Study team members were blinded to febrile hospital participant RBT results and the semi-randomised approach list generation process. A study team member was provided with the approach list and the first febrile hospital participant (or parent/guardian) was approached by phone call so as to obtain verbal consent to visit their household. If a febrile hospital participant declined the household visit or did not answer the phone following three attempts, the study team member recorded the outcome of the approach and moved onto the next febrile hospital participant on the approach list. When the study team member reached the end of the approach list, time-permitting, the study team member would attempt to contact unreachable febrile hospital participants once more. Household visits were arranged between 1 to 10 days following febrile hospital participant enrolment.

### 3.2.9 Selection of household members

All household members aged 2 years and above, and who had been resident in the household for at least 2 months, were eligible for participation in the study.

On arrival at a household, the head of the household was identified and provided
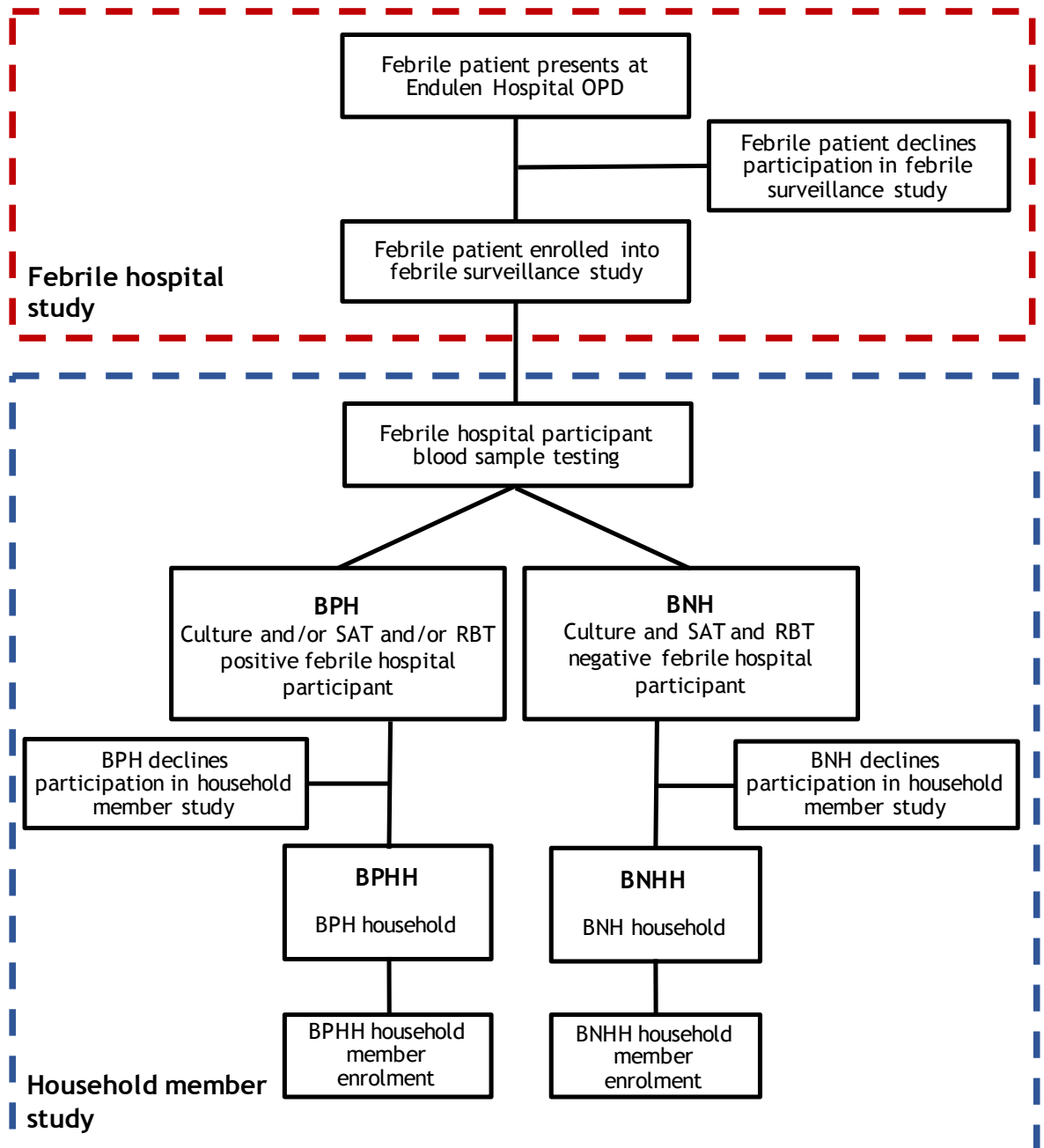


**Figure 3.2: Flow diagram showing identification of BPHs and BNHs, BPHHs and BNHHs and household members for the household member study**: *OPD is outpatient department; RBT is the Rose Bengal plate test; SAT is the serum agglutination test; BPH and BNH are brucellosis positive and negative febrile hospital participants, respectively; BPHH and BNHH are BPH and BNH households, respectively*

with information on the study. Following verbal consent from the head of the household, study participant information was then communicated to all present household members by a study team member. Household members that met the aforementioned eligibility criteria, were read the study participant information

sheet (see Appendix 3 for the Participant Information Sheet). Those individuals agreeing to study participation provided written consent and were enrolled into the study. Household members less than 18 years of age were classified as children and could participate in the study if consent was provided by a parent or guardian (see Appendix 3 for Consent Sheets). Due to sampling time constraints, household participant enrolment was limited to the first six eligible household members that consented to study enrolment.

## 3.2.10 Household participant sampling

Each household participant was weighed, had their tympanic temperature recorded and was asked about any history of fever in the previous 72 hours by a study team member. A structured, closed-ended questionnaire was administered for each participant. Questionnaire topics included: demographic data; current and recent illness symptoms; and reported history of brucellosis. The questionnaire was the same as that used for febrile hospital participants in Chapter 2 (see Appendix 2).

For household participants with a tympanic temperature ≥38 °C at the time of sampling, or a history of fever in the past 72 hours, blood was drawn for culture and serology by a clinical study team member. For household participants weighing ≥25 kg, a 40 mL blood volume was collected. Three culture bottles were inoculated with a target blood volume of 10 mL each: two BacT/ALERT (BioMérieux, Durham, NC, USA) aerobic culture bottles for automated culture; and a Castañeda (Ruiz Castañeda., 1961) bi-phasic media bottle. A plain vacutainer (BD, Franklin Lakes, NJ, USA) was inoculated with a target 10 mL of blood for serological testing. Household participants weighing <25 kg had a blood draw volume calculated based on weight. Sample bottle inoculation was the same as above, except that two paediatric BacT/ALERT bottles were inoculated for automated culture.

For household participants with a tympanic temperature of <38°C at the time of sampling, and not reporting a history of fever in the past 72 hours, a 10 mL blood sample was drawn for serology and inoculated into a plain vacutainer. These non-febrile household participants were not sampled for blood culture

because without evidence of current or recent fever, it was unlikely that the participant had a bloodstream infection and would therefore have a low probability of testing culture positive.

All blood sampling and sample bottle inoculation for culture and serology followed the same protocols as the febrile hospital study (see Chapter 2 Section 2.2.4). Blood culture bottles were inoculated at the household and immediately put on ice until return of the study team to the Endulen Hospital. Plain vacutainer tubes for serology were inoculated and were stood to clot at the household. Vacutainer tubes were then put on ice and transported with the study team to the Endulen Hospital.

### 3.2.11 Sample processing and laboratory diagnostics

On return to the Endulen Hospital, inoculated culture bottles were packed at 2-8°C for transport and transferred to the Kilimanjaro Clinical Research Institute (KCRI), Moshi, Tanzania. Inoculated blood culture bottles arrived at KCRI within approximately 48 hours of sample collection. Both BacT/ALERT and Castañeda blood culture bottles were processed at KCRI, following the same standardised protocols as the febrile hospital study.

Serum was separated at the Endulen Hospital, pipetted into cryovials, put on ice and transported with the inoculated culture bottles to KCRI. Sera were stored at KCRI at -80°C. Sera were tested by RBT, at the Endulen Hospital or at KCRI, using the same testing protocol as described in Section 3.2.4. When the field data collection was complete, all sera were shipped to the APHA, Weybridge, UK for serology testing by SAT.

### 3.2.12 Power analysis

A power analysis was performed in order to estimate the sample size required to detect a difference in prevalence between household participants of brucellosis case and non-case febrile hospital participants. A community brucellosis baseline seroprevalence of 3.4% was assumed based on the results from two cross-sectional surveys using RBT in pastoral areas of northern Tanzania (Halliday JEB, unpublished data). This baseline estimate was used as the estimate for

seroprevalence in BNHH household members. Due to a lack of similar household member screening studies in Africa, seroprevalence estimates from comparable studies in Eastern Europe, the Middle East and Latin America were used to estimate expected brucellosis seroprevalence in household members of brucellosis patients, which would represent the expected seroprevalence in BPHH household members. Examples of such studies outside Africa have estimated seroprevalence between 7% and 20% in brucellosis patients (Alsubaie et al., 2005; Ismayilova et al., 2013; Mendoza-Nunez et al., 2008; Sanodze et al., 2015; Sharifi-Mood et al., 2007; Sofian et al., 2013; Tabak et al., 2008). Based on these empirical data a sample size was selected (n = 40 BPHH household members, n = 200 BNHH household members) that would enable detection of a prevalence difference of at least 14%, with alpha = 0.05, power = 80%. Power analysis was performed using G*Power software version 3.1 (Faul et al., 2009).

### 3.2.13 Statistical analyses

A Bernoulli distributed generalised linear mixed-effect model (GLMM) was fitted to evaluate the relationship between the *Brucella* spp. exposure status of household members and febrile hospital study participants. The response variable was household participant *Brucella* spp. exposure status (positive or negative), as measured by culture and/or SAT and/or RBT. The explanatory variable was household status of the household participant (BPHH or BNHH, defined by the *Brucella* spp. exposure status of the febrile hospital participant). An individual household identifier variable was fitted as a random effect so that autocorrelation in the data caused by multiple household participants screened from the same household could be accounted for. This model was used to evaluate evidence of any grouping of household participant *Brucella* spp. exposures dependent on the brucellosis status of the febrile hospital participant from that household.

A Bernoulli distributed generalised linear model (GLM) was used to investigate and compare the age and sex distributions of household participants from both hospital and household study populations. The model response variable was the study population (febrile hospital study participants versus household member

study participants), and the explanatory variables were participant sex and age in years.

Finally, a second Bernoulli distributed GLM was used to investigate any correlation between the age and sex distributions of *Brucella* spp. exposed household participants, as compared to *Brucella* spp. exposed febrile hospital participants. For this evaluation, the response variable was *Brucella* spp. exposure as defined by RBT in the two study populations (febrile hospital study participants versus household member study participants). The explanatory variables were participant sex and age in years.

A p value ≤0.05 was considered statistically significant across analyses. All data analysis and visualisation was performed in R software version 3.6.1 (R Core Team, 2019) using the *lme4* R package (Bates et al., 2019) and *ggplot2* R package (Wickham et al., 2019).

## 3.2.14 Research clearance and ethics

Implementation of the household member study was approved by the Tanzania Commission for Science and Technology, the Tanzania Wildlife Research Institute and the Ngorongoro Conservation Area Authority. The Kilimanjaro Christian Medical Centre Ethics Committee (698), the National Institute of Medical Research Tanzania (NIMR/HQ/R.8c/Vol.I/1140), and the University of Glasgow College of Medical, Veterinary and Life Sciences Human Ethics Committee (200140149) gave ethical approval for this study. All research conducted was in accordance with the guidelines and regulations of the aforementioned organisations.

## 3.3 Results

### 3.3.1 Febrile hospital study participants

The household member study was conducted from the 5<sup>th</sup> April to 11<sup>th</sup> October 2017. During this time, 114 febrile hospital participants were enrolled into the febrile hospital study. A total of 113 (99.1%) of 114 febrile participants had blood culture and serum samples collected.

Five (4.4%) of 113 febrile hospital participants were culture positive and classified as confirmed acute brucellosis. One (0.9%) of 113 febrile hospital participants showed a four-fold increase in SAT titre and was also classified as confirmed acute brucellosis. Three (2.7%) of 113 febrile hospital participants had a SAT titre ≥160 and were classified as probable acute brucellosis. A total of 109 (96.5%) of 113 febrile hospital participants had serum available for RBT. Thirteen (11.9%) of 109 febrile hospital participants tested RBT positive. Overall, 14 (12.4%) of 113 febrile hospital participants were positive by culture and/or SAT and/or RBT and were classified as a BPH. The remaining 99 (87.6%) febrile hospital participants that were culture, SAT and RBT negative were classified as a BNH.

A total of 103 (91.2%) of the 113 febrile hospital participants were approached for household member sampling. Forty-five (43.7%) of 103 febrile hospital participants consented to household follow-up. Five (11.1%) out of 45 households sampled were classified as BPHH, four of which had acute brucellosis case BPHs and one was BPH by an RBT-identified *Brucella* spp. exposure, see Table 3.1. The remaining 40 households were households of BNHs (febrile hospital participant that was culture, SAT and RBT negative), these households were therefore classified as BNHH, see Figure 3.3 for the household identification process.

**Table 3.1: Brucellosis status as defined by diagnostic test outcome for brucellosis positive febrile hospital participants (BPH)**, *where RBT is Rose Bengal plate test, SAT is serum agglutination test*

| BPH | RBT positive | SAT positive (≥160 titre) | Blood culture positive | Brucellosis status |
|---|---|---|---|---|
| A | Y | Y | Y | Acute brucellosis (confirmed) |
| B | N | N | Y | Acute brucellosis (confirmed) |
| C | Y | Y | N | Acute brucellosis (probable) |
| D | Y | Y | N | Acute brucellosis (probable) |
| E | Y | N | N | *Brucella* spp. exposure |
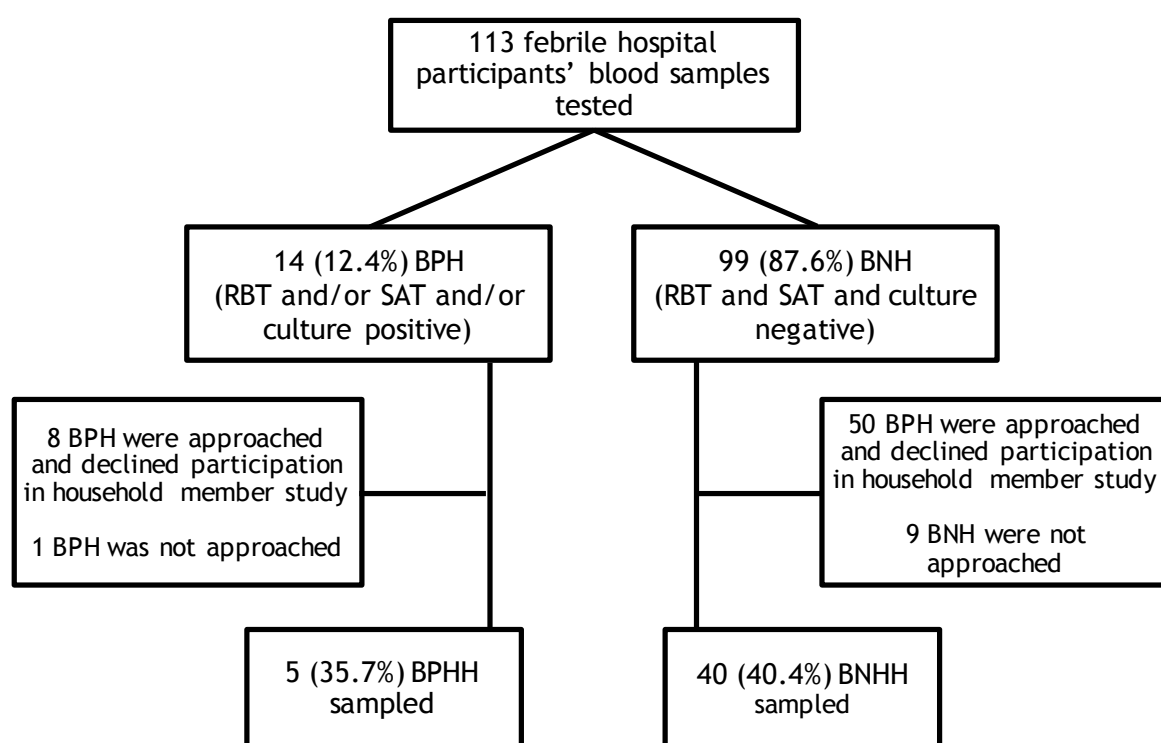


**Figure 3.3: Flow diagram of household member study household identification process**: *BPH and BNH are brucellosis positive and negative febrile hospital participants, respectively; BPHH and BNHH are BPH and BNH households, respectively*

### 3.3.2 Household participant characteristics

Between the 5<sup>th</sup> April 2017 and the 11<sup>th</sup> October 2017, 180 household participants were enrolled from 45 households. Twenty-two (12.2%) of 180 household participants came from BPHHs and 158 from BNHHs. The achieved sample size was less than that indicated in the power analysis (BPHH n = 40 and BNHH n = 200), due to constraints on sample collection which is elaborated on in

the discussion section. A total of 121 (67.2%) of 180 household participants were female and the median household participant age was 20 years (range: 3 - 80 years). Of those enrolled, 77 (43.0%) of 179 with age data available were children. Ten (5.6%) of 180 household participants had a tympanic temperature ≥38.0°C indicating febrile illness on the day of sampling, one was a BPH household participant and nine were BNH household participants. Household participants without a current fever but reporting a history of fever in the last 72 hours included 56 (32.9%) of 170 household participants with data available, five were from BPHHs and 51 were from BNHHs. The majority of household participants 145 (80.6%) of 180 lived within Endulen village, which is the village where Endulen Hospital is situated. The distribution of household participant characteristics across BPHHs and BNHHs is shown in Table 3.2.

**Table 3.2: Household participant characteristics for brucellosis positive and brucellosis negative febrile hospital participant households (BPHH and BNHH),** *where all household participant characteristics indicate total number of participants that data are available for (N), the number of participants within each factor level (n) and the proportion for each factor level (%), with the exception of Age in years where the median and range age is reported for BPH and BNH household participants*

| Participant characteristics | | BPHH household participant n/N (%) | BNHH household participant n/N (%) |
|---|---|---|---|
| Age in years, median (range) | | 24.5 (7, 80) | 19 (3, 80) |
| Sex | Female | 15/22 (68.2) | 106/158 (67.1) |
| | Male | 7/22 (31.8) | 52/158 (32.9) |
| Location | Endulen village | 22/22 (100) | 123/158 (77.8) |
| | Other | 0/22 (0.0) | 35/158 (22.2) |
| Current fever | No | 21/22 (95.5) | 149/158 (94.3) |
| | Yes | 1/22 (4.5) | 9/158 (5.7) |
| History of fever | No | 16/21 (76.2) | 98/149 (65.8) |
| | Yes | 5/21 (23.8) | 51/149 (34.2) |

### 3.3.3 Aim 1: Determining if new acute brucellosis cases could be identified amongst the household members of febrile hospital participants with acute brucellosis

A total of 66 (36.7%) of 180 household participants had a current temperature of ≥38.0°C at the time of household visit or reported a history of fever (within the past 72 hours) and were therefore eligible for blood culture. Sixty-five (98.5%) of 66 household participants had one or more blood culture bottle filled. A total of

141 blood culture bottles were inoculated, 107 BacT/ALERT and 34 Castañeda. No *Brucella* spp. or any other bloodstream infections were identified by culture.

All household participants had a serum sample tested by SAT. Two (1.1%) of 180 household participants had a SAT titre ≥160 and were classified as probable acute brucellosis cases. These acute brucellosis cases came from two different BPHHs. The first acute brucellosis case was a 7 year-old male and reported a history of fever in the last 72 hours. The second acute brucellosis case was a 7 year-old female and did not show evidence of current fever or report a recent history of febrile illness.

### 3.3.4 Aim 2: Estimating the prevalence of *Brucella* spp. exposure amongst the household members of febrile hospital study participants

Of 180 household participants, 176 (97.8%) had a serum sample available for RBT. Nineteen (10.8%) of 176 household participants from 13 different households were RBT positive and classified as *Brucella* spp. exposures. Sixteen (84.2%) of 19 *Brucella* spp. exposures came from ten different BNHHs. The remaining three *Brucella* spp. exposures came from three different BPHHs (see Table 3.3). Estimated seroprevalence by RBT and 95% confidence intervals for BPHH and BNHH household participants, as well as RBT seroprevalence estimates and 95% confidence intervals for four comparison human brucellosis studies in Tanzania are shown in Figure 3.4.

Combining both SAT and RBT results, 21 of 180 household participants tested seropositive, indicating a seroprevalence of 11.7% across household participants. Separating the results into BPH and BNH household participants, seroprevalence was 22.7% and 10.1%, respectively by SAT and RBT (see Table 3.3). Regarding household-level SAT and RBT serostatus, 3 (60.0%) of 5 BPHHs had one or more seropositive household participant, and 10 (25.0%) of 40 BNHHs had one or more seropositive household participant (see Table 3.3). A summary of household participant enrolment and brucellosis test results is given in Figure 3.5.

**Table 3.3: Seroprevalence at the individual household participant-level and household-level as indicated by the Rose Bengal plate test (RBT) and the serum agglutination test (SAT):** *N is the total number of household participants that serology test data are available for; n is the number of seropositive household participants; CI is confidence intervals; and BPHH and BNHH are the brucellosis positive and brucellosis negative febrile hospital participant households, respectively; confidence intervals are reported to 2 decimal places*

| Serological test | BPHH household participant seroprevalence | | BNHH household participant seroprevalence | | BPHH with ≥1 seropositive household participant | | BNHH with ≥1 seropositive household participant | |
|---|---|---|---|---|---|---|---|---|
| | n/N | % (95% CI) | n/N | % (95% CI) | n/N | % (95% CI) | n/N | % (95% CI) |
| SAT | 2/22 | 9.1 (1.12 – 29.16) | 0/158 | 0.0 (0.00 – 2.31) | 2/5 | 40.0 (5.27 – 85.34) | 0/40 | 0.0 (0.00 – 8.81) |
| RBT | 4/22 | 18.2 (5.19 – 40.28) | 16/154 | 10.4 (6.06 – 16.32) | 3/5 | 60.0 (14.66 – 94.73) | 10/40 | 25.0 (12.69 – 41.20) |
| SAT & RBT | 5/22 | 22.7 (7.82 – 40.65) | 16/158 | 10.1 (5.90 – 15.92) | 3/5 | 60.0 (14.66 – 94.73) | 10/40 | 25.0 (12.69 – 41.20) |
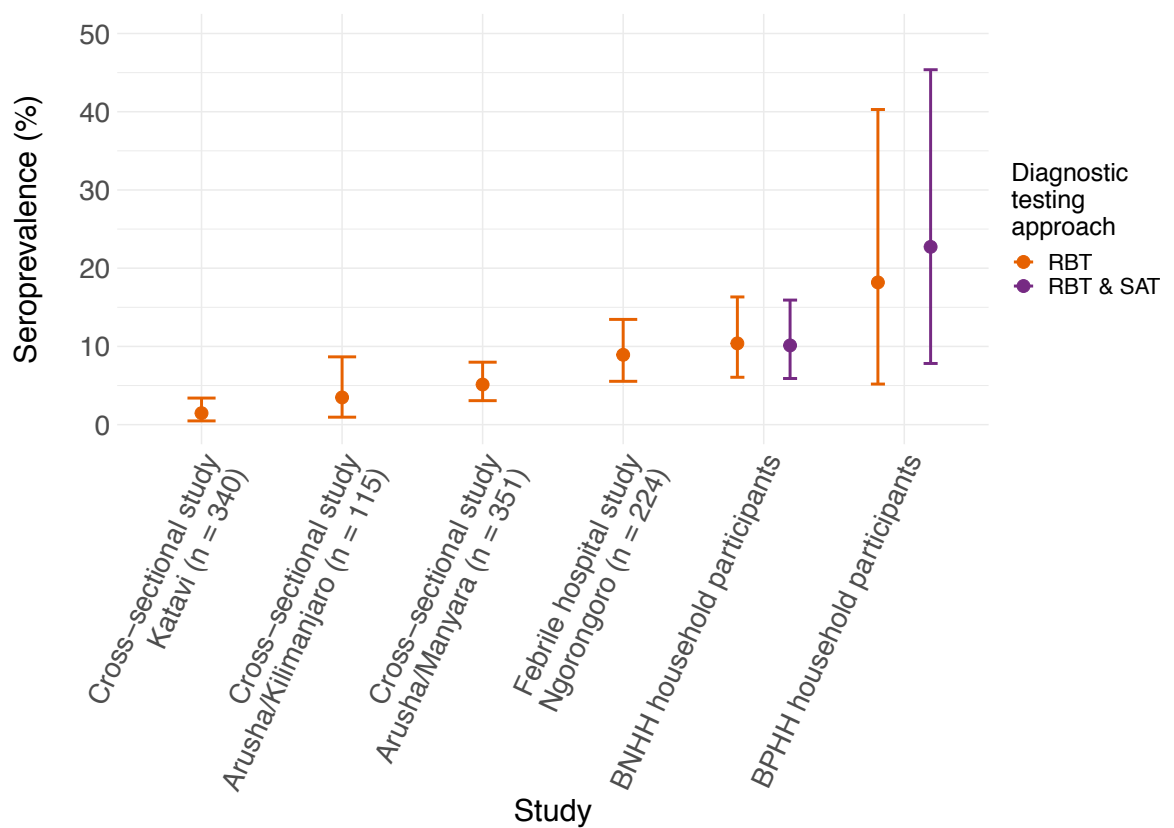
**Figure 3.4: Mean seroprevalence estimates and 95% confidence intervals for human brucellosis studies conducted in agro-pastoral and/or pastoral areas of Tanzania**: *points represent mean seroprevalence estimates per study and bars represent 95% confidence intervals; RBT is the Rose Bengal plate test; SAT is the serum agglutination test; all cross-sectional studies refer to sampling of livestock-keeping households, cross-sectional study Katavi is Assenga et al., 2015; cross-sectional study Arusha/Kilimanjaro and Arusha/Manyara are Halliday JEB, unpublished data; febrile hospital study Ngorongoro is Bodenham et al., 2020; BNHH household participants are household participants in the current study from brucellosis negative hospital participant households, for RBT tested BNHH household participants n = 154, for RBT & SAT tested BNHH household participants n = 158; BPHH household participants are household participants in the current study from brucellosis positive hospital participant households, for RBT tested and RBT & SAT tested BPHH household participants n = 22*
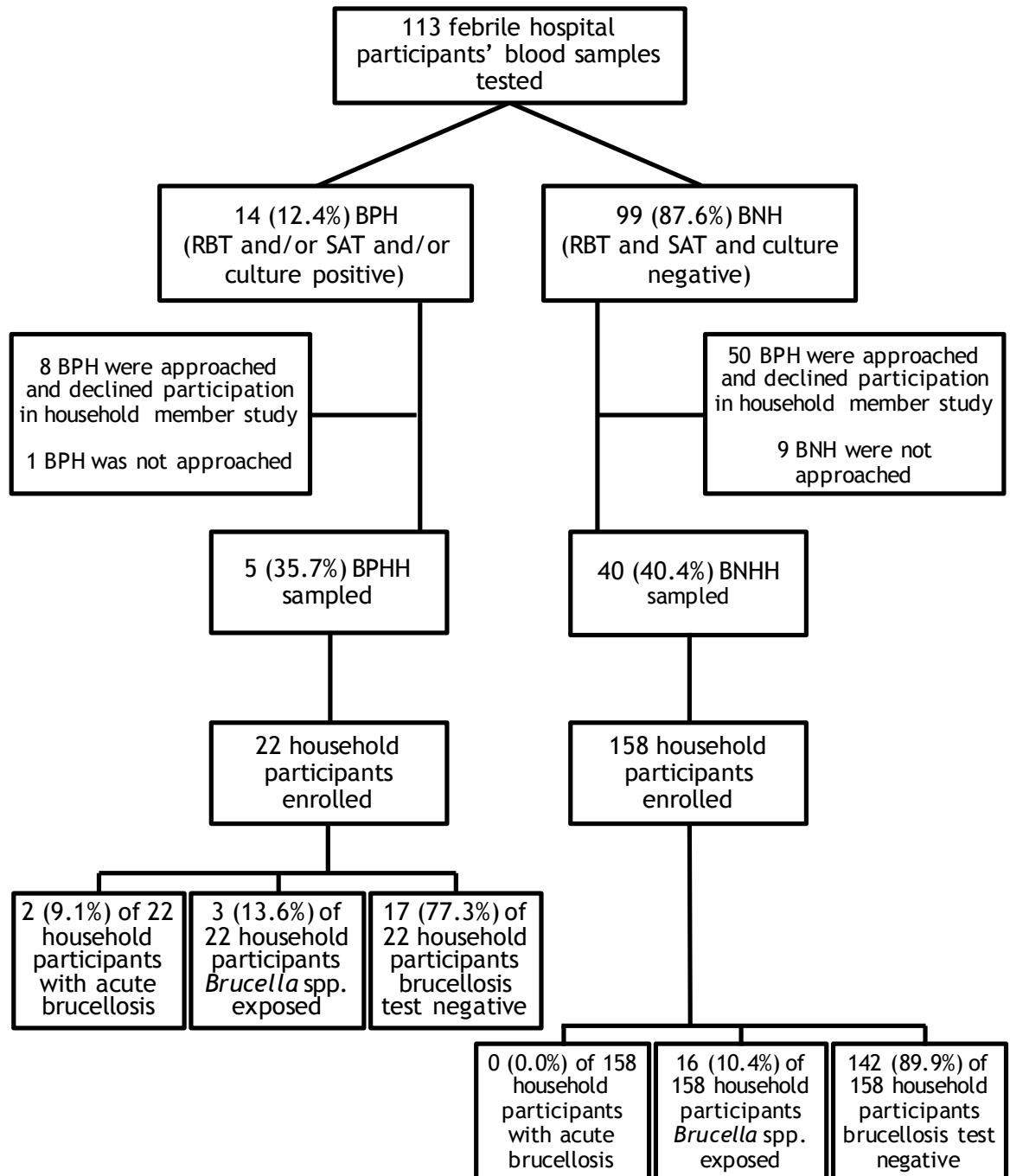
**Figure 3.5: Flow diagram of household member study participant enrolment and brucellosis test results**: *BPH and BNH are brucellosis positive and negative febrile hospital participants, respectively; BPHH and BNHH are BPH and BNH households, respectively*

### 3.3.5 Aim 3: Evaluating evidence of association between *Brucella* spp. exposure status of household participants and febrile hospital participants

A GLMM analysis to evaluate any association between *Brucella* spp. exposure status of household participants and febrile hospital participants was performed. Household status (BPHH or BNHH) was included in the model as the explanatory variable, a unique household identifier was included as a random effect and household participant *Brucella* spp. exposure status was the response variable. There was no significant association between the *Brucella* spp. exposure status of household participants and febrile hospital participants. GLMM estimates are found in Table 3.4.

**Table 3.4: Generalised linear mixed-effect model evaluating association between *Brucella* spp. exposure status of household participants and febrile hospital participants**, *OR is odds ratio, CI is confidence intervals, p value reported to three decimal places, sd is standard deviation*

| Variable | | OR (95% CI) | *p* value |
|---|---|---|---|
| Intercept | | | |
| | | 4.45 (0.01 – 31.61) | <0.001 |
| Household status | BNHH | Ref | |
| | BPHH | 0.04 (0.53 – 68.82) | 0.170 |
| Random effect: Household ID (Variance = 2.51, sd = 1.59, no. of groups = 45) | | | |

### 3.3.6 Aim 4: Comparing the age and sex distributions of febrile hospital study and household member study participants

A total of 228 (98.2%) of 232 febrile hospital participants enrolled during the febrile hospital study had age and sex data available. Of household participants, 179 (99.4%) of 180 had age and sex data available. Age and sex distributions for the two study populations are shown in Figure 3.6. Febrile hospital participants had a median age of 27 years (range: 2-78 years). Household participants had a median age of 20 years (range: 3-80 years). A total of 129 (56.6%) of 228 febrile hospital participants were female and 120 (67.0%) of 179 household participants were female.

Multivariable GLM analysis to compare the febrile hospital study versus household member study (reference population) participant sex and age (years) distributions was performed. Analyses show that study population was significantly associated with: sex (LRT $\chi_2$ = 5.00, df = 1, p = 0.025, n = 407), with participants in the febrile hospital study having higher odds of being male as compared to participants in the household member study (aOR = 1.59, 95% CI = 1.06 – 2.41, p = 0.026); and age (LRT $\chi_2$ = 4.64, df = 1, p = 0.031, n = 407), with participants in the febrile hospital study having higher odds of increasing age in years as compared to participants in the household member study (aOR = 1.01, 95% CI = 1.00 – 1.02, p = 0.034), see Table 3.5 for further details.
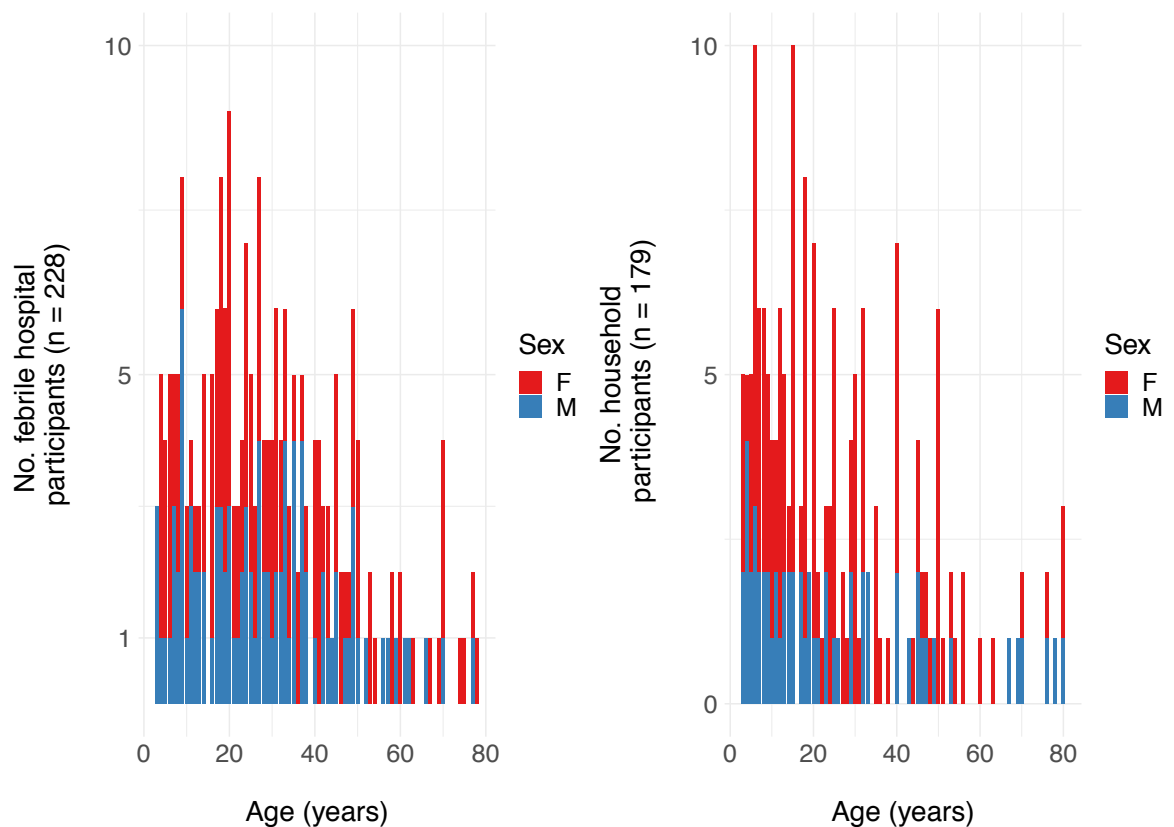


**Figure 3.6: Age and sex distribution of febrile hospital study participants (left panel) and age and sex distribution of household member study participants (right panel)**

**Table 3.5: Multivariable generalised linear model evaluating the relationship between febrile hospital participant versus household member participant (reference population) study populations and the covariables sex and age in years**, *aOR is adjusted odds ratio, CI is confidence interval, LRT is likelihood ratio test, χ2 is chi-squared test statistic, p values are reported to three decimal places*

| Variable | | aOR (95% CI) | aOR *p* value | LRT $\chi_2$ | LRT *p* value |
|---|---|---|---|---|---|
| Intercept | | | | | |
| | | 0.77 (0.52 – 1.1) | 0.196 | | |
| Sex | Female | Ref | | | |
| | Male | 1.59 (1.06 – 2.41) | 0.026 | 5.00 | 0.025 |
| Age (years) | | | | | |
| | | 1.01 (1.00 – 1.02) | 0.034 | 4.64 | 0.031 |

### 3.3.7 Aim 5: Comparing the age and sex distributions of RBT-defined *Brucella* spp. exposed febrile hospital study and household member study participants

RBT was performed on 224 (96.6%) of 232 febrile hospital participants with serum available and 176 (97.8%) of 180 household participants with serum available. Twenty (8.9%) of 224 febrile hospital participants were RBT positive. Twenty (11.4%) of 176 household participants were RBT positive. Age and sex distributions for RBT-defined *Brucella* spp. exposed febrile hospital participants and household participants are shown in Figure 3.7. Of 20 RBT positive febrile hospital participants, the median age was 13.5 years (range: 7-62 years). Of twenty RBT positive household participants, the median age was 23 years (range: 7-80 years). Seven (35.0%) of 20 RBT positive febrile hospital participants were female. Fourteen (70.0%) of 20 RBT positive household participants were female.

A GLM analysis comparing RBT-defined *Brucella* spp. exposed febrile hospital study versus household member study participant (reference population) sex and age (years) distributions was performed. The most parsimonious final model included the variable sex (LRT $\chi_2$ = 5.019, df = 1, p = 0.025, n = 40), with *Brucella* spp. exposed participants in the febrile hospital study having higher

odds of being male as compared to *Brucella* spp. exposed participants in the household member study (OR = 4.33, 95% CI = 1.20 – 17.43, p = 0.030), see Table 3.6.
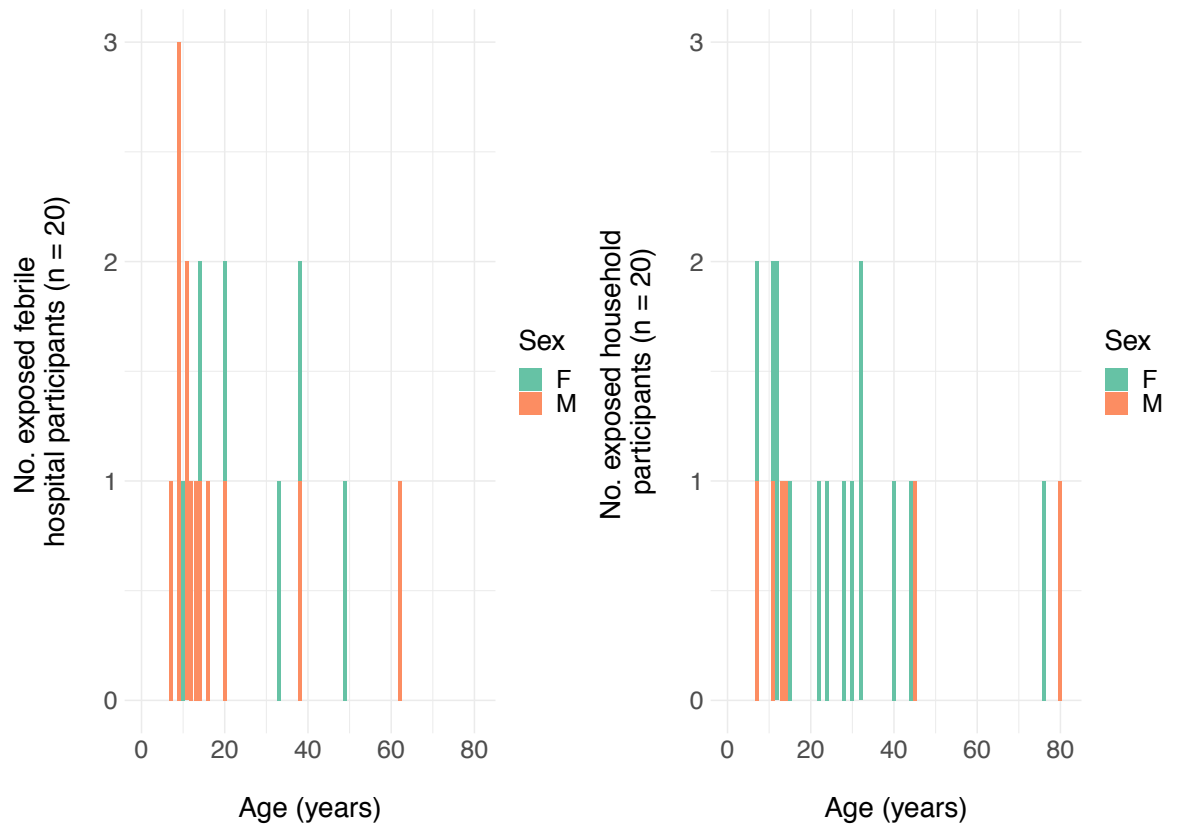


**Figure 3.7: Age and sex distribution of febrile hospital study RBT-defined Brucella spp. exposed participants (left panel) and age and sex distribution of household member study RBT-defined Brucella spp. exposed participants (right panel)**, *RBT is the Rose Bengal plate test*

**Table 3.6: Maximal and final generalised linear models evaluating relationship between RBT-defined *Brucella* spp. exposed participants in the febrile hospital study versus household member study (reference population) and the covariables sex and age (years)**, *aOR is adjusted odds ratio, CI is confidence interval, LRT is likelihood ratio test, $\chi_2$ is chi-squared test statistic, p values are reported to three decimal places*

| Variable | | Maximal model aOR (95% CI) | aOR $p$ value | LRT $\chi_2$ | LRT $p$ value | Final model OR (95% CI) | OR $p$ value | LRT $\chi_2$ | LRT $p$ value |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | | 0.82 (0.22 – 3.05) | 0.771 | | | 0.50 (0.19 – 1.20) | 0.134 | | |
| Sex | Female | Ref | | | | Ref | | | |
| | Male | 4.05 (1.09 – 16.59) | 0.042 | 4.36 | 0.037 | 4.33 (1.20 – 17.43) | 0.030 | 5.02 | 0.025 |
| Age (years) | | 0.98 (0.94 – 1.02) | 0.304 | 1.09 | 0.295 | | | | |

## 3.4 Discussion

This is the first study in Tanzania, and possibly in SSA, to investigate brucellosis active surveillance in the form of active case finding in household members of febrile hospital patients. Two household participants were identified as acute brucellosis cases in two separate BPHHs, no acute brucellosis case was identified in BNHHs. Seroprevalence in household participants from BPHHs and BNHHs was estimated at 22.7% (95% CI: 7.82 – 40.65) and 10.1% (95% CI: 5.90 – 15.92), respectively. Three of 5 BPHHs and 10 of 40 BNHHs had one or more seropositive household participant. In the GLMM analysis, there was no significant association between the *Brucella* spp. exposure status of household participants and febrile hospital participants. A GLM comparing the age and sex distributions of the febrile hospital study participants and the household member study participants indicated a significant association with age and sex. Being male and of increasing age in years was significantly associated with febrile hospital participants. A further GLM comparing the age and sex distributions of *Brucella* spp. exposed (as measured by RBT) febrile hospital participants and household participants showed an association with sex. Being male was significantly associated with *Brucella* spp. exposed febrile hospital participants.

This household member study detected two acute brucellosis cases in the household participants of two separate BPHHs. The identification of acute brucellosis cases in household members that have not visited hospital suggests that hospital-based surveillance alone will underestimate acute brucellosis burden. Although this is a small sample size, this finding is consistent with the hypothesis that there are shared risk factors for *Brucella* spp. infections at the household-level (Tabak et al., 2008). In Chapter 2, low age was identified as a risk factor for acute brucellosis in febrile hospital participants, with acutely infected individuals having a median age of 11 years (range: 7-20 years). Here, both acute brucellosis household participants were 7 years-old and therefore fit into the pre-identified age range for acute brucellosis cases in the febrile hospital population. Having herded any livestock over the past 12 months was also a risk factor for acute brucellosis in febrile hospital participants in Chapter 2. Here, one of the two household participants identified as a probable acute brucellosis case reported having herded livestock.

The seroprevalence of *Brucella* spp. exposure in household participants from BPHHs was 22.7% and was higher than seroprevalence in household participants from BNHHs estimated as 10.1%. However, the confidence intervals for these estimates do substantially overlap. Other studies in Eastern Europe and the Middle East have estimated seroprevalence between 7% to 20% for household members of brucellosis patients (Almuneef et al., 2004; Alsubaie et al., 2005; Ismayilova et al., 2013; Sanodze et al., 2015; Sharifi-Mood et al., 2007; Sofian et al., 2013; Tabak et al., 2008). The aforementioned studies do differ in study design and in geographical setting. They are therefore not directly comparable to the present study, but they do however give an indication of an approximate seroprevalence range in the absence of an evident comparable study in SSA.

There are examples of cross-sectional studies that have used RBT to investigate seroprevalence of *Brucella* spp. exposure in agro-pastoralist and pastoralist households in Tanzania. These human seroprevalence estimates ranged from 1.5% to 5.1% (Assenga et al., 2015) (Halliday JEB, unpublished data). In the linked febrile hospital study, seroprevalence of brucellosis in febrile hospital participants as defined by RBT was 8.9%. In the current study, considering RBT-defined seroprevalence estimates only so as to compare to previous studies in similar settings in Tanzania, BPHH and BNHH seroprevalence was 18.2% (95% CI: 5.19 – 40.28) and 10.4% (95% CI: 6.06 – 16.32), respectively. The higher seroprevalence estimates (albeit with wide confidence intervals) in this study are not surprising considering the difference in the sampled population as compared to the other study examples. For example, the studies differed in: surveillance location (household, hospital); health status of participant (febrile, non-febrile); geographic location; and livestock-keeping practices (pastoral, agro-pastoral).

The GLMM was fitted to investigate any association between the *Brucella* spp. exposure status of household participants and febrile hospital participants. The study sample size achieved was ultimately not sufficient to power this analysis investigating evidence of grouping in *Brucella* spp. exposures and no significant association was identified. However, a trend towards an increased number of

*Brucella* spp. exposed household participants in BPHHs was observed, although this was not significant.

The sex and age distributions of the febrile hospital study and household member study populations were compared using a multivariable GLM. There was significant association found for both sex and age distributions in the two study populations. Being male and of increasing age in years was significantly associated with febrile hospital participants. The sex and age distributions of RBT-defined *Brucella* spp. exposed febrile hospital and household participants were also compared by multivariable GLM. The final model identified that being male was significantly associated with *Brucella* spp. exposed febrile hospital study participants as compared to household study participants. It should be highlighted that the two study populations being compared here were: febrile hospital patients; and household members of febrile hospital participants. The selection steps for these two populations were different and therefore the results from these analyses should be interpreted with caution. Comprehensive studies investigating gender differences in disease burden such as for tuberculosis and HIV in LMICs have found that males are relatively disadvantaged in accessing healthcare (Auld et al., 2015; Horton et al., 2016). It is possible that being female and of decreasing age in years, and equally being female and *Brucella* spp. exposed, was associated with enrolment in the household member study because younger females were relatively more likely to be present during household member sampling. Of all study participants enrolled in the household member study, 67.2% were female. Males may have been more likely to be absent from the household due to livestock-keeping or employment responsibilities. For the febrile hospital study, 56.6% of all participants were female. However, 75.0% of RBT-defined *Brucella* spp. exposures were male, indicating a relatively higher *Brucella* spp. seroprevalence in male febrile hospital study participants. Similarly, regarding the brucellosis case data for the febrile hospital study, 71.4% of brucellosis cases (defined but culture or SAT) were male (Bodenham et al., 2020). Overall, these findings suggest that both males and females are at risk of *Brucella* spp. exposure, and there are varying factors influencing male and female enrolment in both healthcare facility and community-based surveillance approaches. These findings reinforce that analysis

of hospital-based data alone will likely not be representative of the true disease burden in the community.

A limitation of this study was that the household member sample size estimated by the power analysis was not achieved. Therefore, a lack of sufficient power may be the reason why there was no significant association between household participant and febrile hospital participant *Brucella* spp. exposure status. Meaning that grouping of exposures to *Brucella* spp. at the household-level was not found. There were different study challenges that negatively impacted on the number of samples collected. Firstly, the household study sample size pool was limited by the total number of febrile hospital participants enrolled (n = 113), during the household study timeframe. It is important to acknowledge that this study was also subject to selection bias as the household participation decline rate was 56.3%. Reasons for study participation decline included the highly mobile nature of the study community, and the invasive sampling requested. Those households that did participate may have had a head of household that was more highly educated and more aware of brucellosis. Participating households may have been at a lower risk of exposure to *Brucella* spp. due to this disease awareness, interest in family health, and reduced household mobility, as compared to the non-participating, more mobile households. Additionally, household members sampling was not randomised; the first six household members that consented to participate were sampled. This approach may have resulted in more influential household members self-selecting which individuals would be sampled and potentially prioritising those individuals with a history or suspected brucellosis, meaning that a true representation of household member health was less likely to be achieved. Therefore, selection biases may have resulted in underestimation of the prevalence of brucellosis in the household members of febrile hospital participants due to a high household decline rate, or overestimated prevalence due to non-randomised household member sampling and the potential for preferential selection of sick household members.

As a national active surveillance tool, screening of household members of febrile hospital patients may be too resource-intensive to justify its routine implementation in Tanzania. However, further study may benefit from

increasing the duration of a household-based surveillance research, alongside the continuation of hospital-based surveillance. This would improve power to detect any grouping of *Brucella* spp. exposures in the households of febrile hospital participants, if truly present. The availability of these *Brucella* spp. exposure data from community members that do not reach a healthcare facility are important in informing effective, targeted implementation of brucellosis prevention and control interventions.

## 3.5 Conclusion

Active surveillance in the form of screening household members of brucellosis patients has been suggested for areas endemic to brucellosis (Moreno, 2014; Tabak et al., 2008), and in combination with routine passive surveillance can help in understanding the true burden of brucellosis (Mantur and Amarnath, 2008). Examples of this form of active case finding surveillance are hard to find in SSA, however studies have indicated their necessity in this region (Asiimwe et al., 2015; Migisha et al., 2018). In the current study, acute brucellosis cases were detected in household participants of BPHHs. Despite the number of additional acute brucellosis cases detected being small, this method of targeted active surveillance indicates that the use of hospital-based surveillance alone will underestimate the true brucellosis burden. The seroprevalence estimate was highest for household participants of BPHHs as compared to BNHHs. However, no significant association was identified between *Brucella* spp. exposures in household participants and febrile hospital participants. This is likely due to the challenges of data collection of this type and consequent limited power for this analysis. Active surveillance by screening household members requires further study but may prove too resource-intensive for routine implementation. However, it could provide valuable data on disease burden for the population that do not reach a healthcare facility, as well as assist in targeting prevention and control measures towards high-risk populations in Tanzania and other comparable areas in SSA.

# Chapter 4 Evaluating the performance of serological tests in detecting animal brucellosis in Tanzania

## 4.1 Introduction

Brucellosis is endemic in many of the pastoral areas of sub-Saharan Africa (SSA) (Mcdermott and Arimi, 2002), where people commonly live in close contact with livestock species (Pappas et al., 2006). Animal reservoirs are the source of human infections (Godfroid et al., 2005). Therefore, the control and prevention of brucellosis in animal host species is a key approach in the control and prevention of human brucellosis (Corbel, 1997; Doganay and Aygen, 2003). The ability to successfully identify animal host species is important in reducing the burden of human brucellosis (Bronsvoort et al., 2009). The application of effective diagnostic tools for the identification of brucellosis in livestock is particularly important in high-risk pastoralist communities. Effective diagnostic tools can assist in understanding the epidemiology of brucellosis in these areas, and these data can be used to guide animal control activities.

Diagnostic test performance is evaluated by assessment of a test's sensitivity and specificity, which pertain to the capacity of a test in indicating the true disease status (Speybroeck et al., 2013). The gold standard diagnostic test for brucellosis is isolation of *Brucella* spp. by culture, which enables confirmation of a positive infection status (Ducrotoy et al., 2018). Although culture specificity is high, sensitivity can be low as *Brucella* spp. are fastidious and may be easily out-competed by contaminating bacteria (Matope et al., 2011). Equally, the type of sample collected for culture and the selective media used can also affect diagnostic sensitivity (Ducrotoy and Bardosh, 2017; Miguel et al., 2011). The high cost of diagnosis by culture, as well as the need for high-security laboratory facilities and bacteriological expertise, make this diagnostic approach largely inaccessible in low- and middle-income countries (LMICs) (Ducrotoy and Bardosh, 2017). Serological tests are easier to implement than bacteriological culture and do not require high-security laboratory infrastructure (Díaz et al., 2011), making serology a more feasible routine brucellosis diagnostic approach in LMICs.

Serological tests use blood serum to identify an antibody response to *Brucella* spp. exposure (Ducrotoy and Bardosh, 2017). There are a large number of serological tests available that can be broken down into the following groups: agglutination tests; primary binding assays; precipitation tests; and complement fixation tests (Nielsen, 2002). There is no single serological test that performs with high sensitivity and specificity in all epidemiological situations, nor for all animal species. Therefore, it is recommended that sera be tested by both a recognised screening test and an established confirmatory test to optimise sensitivity and specificity of diagnostic testing (OIE, 2018). For cattle and small ruminants, the Rose Bengal plate test (RBT), is a joint World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and World Organisation for Animal Health (OIE) suitable screening test for animal brucellosis (OIE, 2018). The RBT is an agglutination test that is reported as having a high sensitivity when testing animal samples in field and laboratory settings (Robinson, 2003). Enzyme-linked immunosorbent assay (ELISA) methods are also suitable screening tests in the detection of animal brucellosis (OIE, 2018). The competitive enzyme-linked immunosorbent assay (cELISA) is a primary binding assay that is usually more specific than the RBT and indirect ELISA (iELISA) (Makita et al., 2011a; OIE, 2018). However, cELISA sensitivity may be lower than that of RBT or iELISA (OIE, 2018).

The evaluation of diagnostic tests in the epidemiological setting in which they are implemented is important in understanding setting-specific test performance, especially in the absence of a gold standard approach. The Hui and Walter latent class model can be used to generate estimates of disease prevalence, as well as the sensitivity and specificity of a number of diagnostic tests where no gold standard is available (Hui and Walter, 1980). Test evaluation by latent class analysis requires a minimum of two diagnostic tests in a minimum of two subpopulations (Branscum et al., 2005). There are three model assumptions that should be met: (a) that prevalence is different between each subpopulation; (b) that the diagnostic tests perform the same across subpopulations; and (c) that the diagnostic tests are conditionally independent regarding disease status (Hui and Walter, 1980). Bayesian adaptation of the no gold standard latent class model allows the inclusion of prior knowledge of disease prevalence, as well as sensitivity and specificity of each diagnostic test

being evaluated based on available estimates from previous studies (Branscum et al., 2005). Alternatively, uniform prior distributions may be used if prior information is not available (Branscum et al., 2005). Probabilities of each diagnostic test outcome conditional on an unknown disease status are estimated using the sensitivity and specificity of each diagnostic test and the prevalence of the disease in each subpopulation (Mazeri et al., 2016). Bayesian latent class models can also be expanded to include estimation of test performance under different diagnostic testing approaches, such as in-series and in-parallel testing. These different approaches allow the evaluation of using multiple tests in disease detection. Using an in-series approach with two diagnostic tests, a sample should test positive by both tests to be classified as disease positive. This diagnostic approach has the advantage of increasing the overall specificity of the selected tests but decreases sensitivity (Dohoo et al., 2003b). With an in-parallel approach, all samples are tested by both diagnostic tests and a positive result in either test is classified as a disease positive. This approach improves overall sensitivity but causes a reduction in specificity (Dohoo et al., 2003b).

There are recent examples across Africa for the evaluation of RBT or cELISA performance in diagnosing brucellosis in cattle (Bronsvoort et al., 2009; Chisi et al., 2017; Etman et al., 2014; Getachew et al., 2016; Hosein et al., 2017; Matope et al., 2011; Muma et al., 2007; Sanogo et al., 2013). A handful have assessed both RBT and cELISA (Chisi et al., 2017; Etman et al., 2014; Matope et al., 2011; Muma et al., 2007). A study in Zimbabwe reported relatively high cELISA sensitivity and high RBT specificity for cattle samples, advocating the use of these two tests in combination (Matope et al., 2011). Similar studies in Africa for sheep and goats are not evident, although there are examples for Asia, Europe and the Americas (García-Bocanegra et al., 2014; Nielsen et al., 2005; Rahman et al., 2013; Ramírez-Pfeiffer et al., 2008).

In Tanzania, 40% of the population practices exclusive pastoralism (PINGO's Forum, 2016), and can be considered at high-risk for brucellosis infection. There is currently no national standardised and validated testing procedure for animal brucellosis in Tanzania (Government of Tanzania, 2018a). Therefore, the evaluation of diagnostic test performance and the identification of an evidence-based diagnostic testing approach for animal brucellosis in this context

specifically are vital. The implementation of a standardised and validated testing procedure for animal brucellosis can assist in generating a more robust true burden estimate for brucellosis in Tanzania and the wider SSA. These data can also be used to inform and evaluate evidence-based surveillance and control activities in Tanzania.

Here, the performance of the RBT and cELISA diagnostic tests, including in-series and in-parallel diagnostic testing approaches, were evaluated for cattle, sheep and goats sampled in northern Tanzania using variants of a no gold standard Bayesian adaptation of the Hui-Walter latent class model.

### 4.1.1 Study aims

The aims of this study were:

1 – To estimate the sensitivity and specificity of RBT and cELISA tests for cattle, sheep and goats of northern Tanzania

2 – To estimate the prevalence of brucellosis in cattle, sheep and goats of northern Tanzania for two subpopulations: exclusive pastoralist and non-exclusive pastoralist

3 – To compare these estimates to existing literature estimates for cattle, sheep and goats

4 - To evaluate RBT and cELISA combined test performance when using in-series and in-parallel testing approaches

## 4.2 Methods

The methods detailed in Sections 4.2.1 – 4.2.5 were not conducted as part of this research chapter, but these methodological sections are described so as to understand the source of the data used to inform the latent class analyses performed here. Methods that were performed and contributed specifically to the research outcomes for this chapter include Sections 4.2.6 onwards.

### 4.2.1 Study area

Animal data collection was conducted in three regions of northern Tanzania: Arusha, Manyara and Kilimanjaro. Across these neighbouring regions there are a mix of livestock-keeping systems including exclusive pastoral, agro-pastoral and commercial. The Tanzanian 2016 to 2017 livestock census estimated a cattle population of approximately 1.2 million for Arusha, 2.2 million for Manyara and 794,000 for Kilimanjaro (Government of Tanzania, 2017). For sheep, the approximate population for Arusha was 659,000, Manyara was 444,000 and Kilimanjaro was 182,000 (Government of Tanzania, 2017). From the same census, the goat population was estimated at approximately 2.6 million for Arusha, 1.8 million for Manyara and 664,000 for Kilimanjaro (Government of Tanzania, 2017).

### 4.2.2 Study design

The data used to support the latent class analyses came from two cross-sectional studies. The first study was conducted in Arusha and Kilimanjaro Regions between September 2013 to March 2015, and the second in Arusha and Manyara Regions from January to December 2016.

*Arusha/Kilimanjaro study*: A survey of livestock-owning households was conducted across seven districts in Arusha and Kilimanjaro Regions: Hai; Longido; Monduli; Moshi Municipal; Moshi Rural; Mwanga; and Rombo. A list of the wards within each district was obtained from census records. Wards were first identified as rural or urban based on national census data (NBS, 2013). Urban wards within Hai, Moshi Municipal, Moshi Rural, Mwanga and Rombo districts were classified as peri-urban production areas. Rural wards within Hai, Moshi Municipal, Moshi Rural, Mwanga and Rombo districts that did not contain a

substantial population of pastoralist livestock-keepers were classified as agro-pastoral production areas. Rural wards within Longido and Monduli districts of the Arusha Region that included a substantial population of pastoralist livestock-keepers were classified as pastoral production areas.

A multistage sampling approach was adopted to select wards, villages, sub-villages and livestock-owning households for inclusion in the study. Six wards were selected at random from each production area to give a total of 18 randomly selected study wards. One village or sub-village (depending on the smallest unit applicable) from each ward was randomly selected for inclusion, see Figure 1 for the distribution of villages/sub-villages sampled. Households were randomly selected from a list of livestock-keeping households generated through consultation with local community leaders in each village. A minimum of five households were selected in each village/sub-village. At each household, up to 15 cattle, sheep and goats were randomly selected. In households with more than 15 of each livestock species, adult females were prioritised. Individual-level animal data were collected including: species; age; sex; breed; and vaccination status.

*Arusha/Manyara study:* A survey of livestock keepers was conducted in ten districts in Arusha and Manyara Regions: Longido; Monduli; Arusha; Karatu; Meru; Ngorongoro; Babati Rural; Babati Urban; Mbulu; and Simanjiro. Villages lists were obtained from national census data (NBS, 2013). Villages in wards specified in the census data as urban were excluded from the selection procedure. Villages were classified as: pastoral, where livestock rearing was considered to be the primary livelihood activity; and mixed, where a combination of crop production and livestock keeping were important. Classification of villages was performed in consultation with district government officials. Village selection was stratified by production classification, with 11 pastoral villages and 9 mixed villages selected, see Figure 1 for the distribution of villages sampled.

A multistage sampling approach was used for the selection of households. Each selected village consisted of two to four sub-villages. Two to three sub-villages were randomly selected for sampling in each village. In each selected sub-village, a central point sampling approach was applied, where livestock keepers
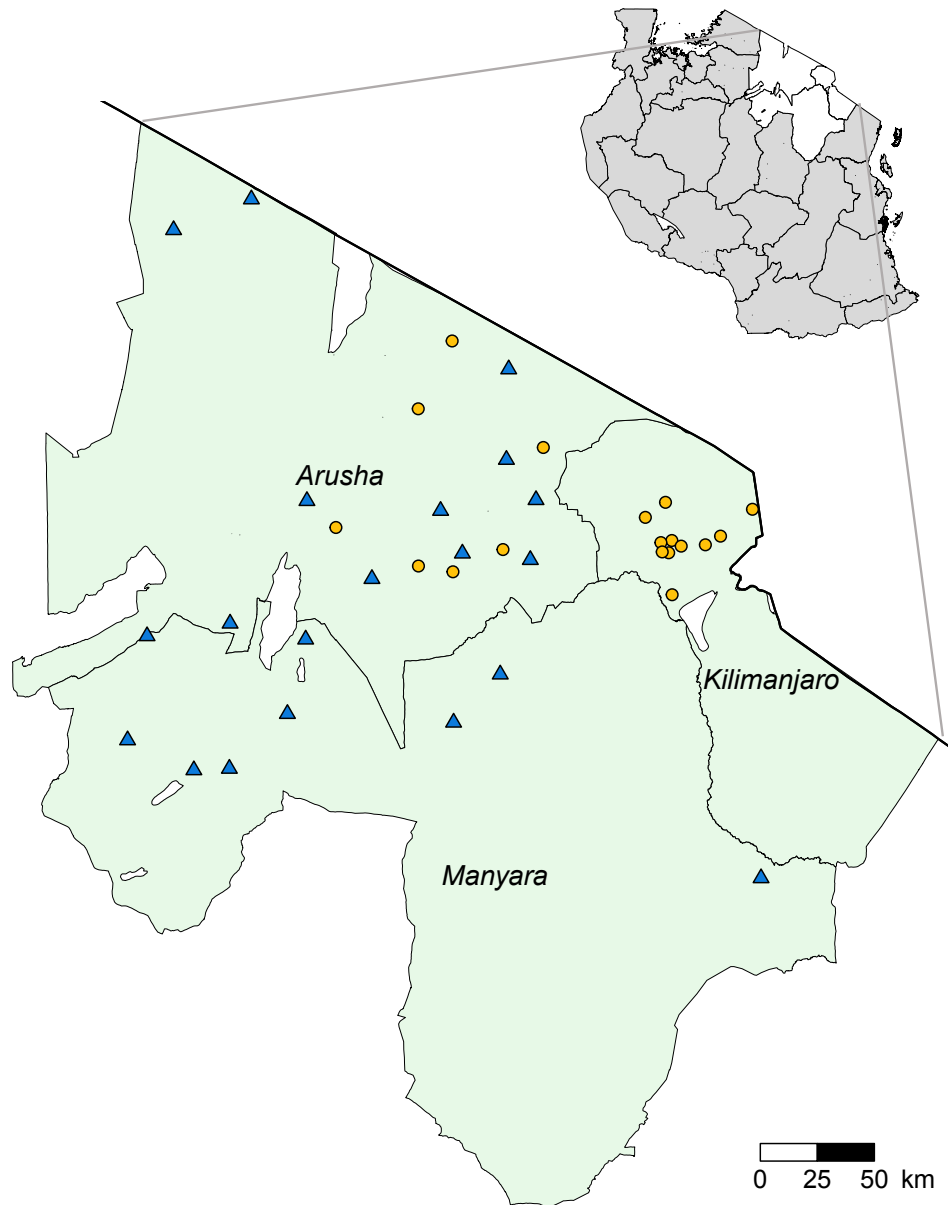
**Figure 4.1: Arusha, Kilimanjaro, and Manyara Regions (green shading) in Tanzania**, *the solid black line represents the border with Kenya, yellow circles show the villages/sub-villages sampled for the Arusha/Kilimanjaro study, blue triangles show the villages/sub-villages sampled for the Arusha/Manyara study. In the top right insert, white polygons show Arusha, Kilimanjaro and Manyara Region locations within an outline map of Tanzania (grey shading). Shapefiles of administrative boundaries from the 2012 census were sourced from the Tanzania National Bureau of Statistics.*

and their animals were invited to a predetermined point within the sub-village. At this central point, a list of the attending households was recorded, and a maximum of ten households selected using a random number generator. From the selected households, a maximum of 10 cattle, 10 sheep and 10 goats were sampled. A target of at least 5 juvenile animals, including 2 juvenile males and 5

adult animals, including 2 adult males were selected at random per species, per household. Individual-level animal data were collected including: species; age; sex; breed; and vaccination status. The full study methodology is described elsewhere (Herzog et al., 2019).

### 4.2.3 Sample collection

Both studies collected up to 10 mL of venous blood into a plain vacutainer (BD, Franklin Lakes, NJ, USA) from all selected livestock. Blood samples were allowed to clot and centrifuged at 1300 g for 10 minutes. Serum was aliquoted into sterile samples tubes in the field and stored at 4 °C in a mobile refrigerator for up to 72 hours before being heat treated at 56 °C for two hours and stored at -80 °C.

All sera were tested by both RBT and cELISA. Based on the absence of reports of any vaccination efforts for *Brucella* in the study regions and no reports of *Brucella* vaccination in the sampled households, detected seropositivity was assumed to reflect natural exposure to *Brucella* spp.

### 4.2.4 Rose Bengal plate test

All sera were tested by the Rose Bengal plate test (RBT) at the field site or in the Kilimanjaro Clinical Research Institute (KCRI) laboratory, Tanzania. The same RBT standard protocol was used as described in Chapter 3, Section 3.2.4., except that the serum to antigen ratio used for each livestock species varied. For cattle samples, the ratio remained the same as in humans, using 25 μL serum to 25 μL antigen. For sheep and goats, the recommended modified serum to antigen ratio, 75 μL serum to 25 μL antigen, was used (OIE, 2018).

### 4.2.5 Competitive enzyme-linked immunosorbent assay

Sera testing by competitive enzyme-linked immunosorbent assay (cELISA) for the 2013 to 2015 study was performed at the Animal and Plant Health Agency (APHA), UK. Diagnostic testing by cELISA for the 2016 study was conducted at KCRI. Cattle, sheep and goat sera testing for both studies was performed using the COMPELISA kit (APHA SCIENTIFIC, Weybridge, UK). cELISA was implemented following the COMPELISA kit instructions as follows: diluting buffer was brought

to ambient temperature and conjugate concentrate was then diluted to working strength. Using a 96-well plate, 20 $\mu$L of each serum sample was pipetted into an individual well. Positive and negative control sera were pipetted at 20 $\mu$L volumes into 5 wells each. Four empty wells acted as conjugate controls. Immediately, 100 $\mu$L of conjugate solution was pipetted into all wells. The plate was then vigorously shaken for two minutes. Next, the plate was covered and incubated at 21°C ± 6°C for 30 minutes on a rotary shaker at 160 revs/min. Following incubation, the plate was inverted, and the solution tapped from the wells. Drinking water was used to wash the plate 5 times and the plate inverted again and dried using absorbent towel until no more liquid could be removed. Prior to use, OPD solution was prepared by dissolving one tablet of urea hydrogen peroxide in 12 mL of distilled water, one OPD tablet was then added to the solution and mixed thoroughly. A 100 $\mu$L volume of OPD solution was pipetted into each well. The plate was then incubated at 21°C ± 6°C for between 10 and 20 minutes. Finally, 100 $\mu$L of stopping solution was pipetted into each well and the plate read on a microplate reader at 450 nm. Positive sera were those lacking colour development. The 4 conjugate control wells were used to calculate 60% of the mean optical density (OD), which was used as the positive/negative cut-off value. Sera with an OD value equal to or less than the cut-off value were classified as positive.

## 4.2.6 Subpopulation classifications

The animal diagnostic test data from both studies were combined and classified as coming from one of two subpopulations: pastoralist, or non-pastoralist. Exclusive pastoralist was defined as: all animals sampled in villages/sub-villages previously identified as predominantly livestock keeping production areas. Non-exclusive pastoralist was defined as: all animals sampled in villages/sub-villages previously identified as a combination of crop and livestock keeping production areas.

## 4.2.7 Statistical analyses: Bayesian Hui-Walter model

A previously described Bayesian version of the no gold standard Hui-Walter model (Bronsvoort et al., 2009; Mazeri et al., 2016; Toft et al., 2005) was used to evaluate performance of the RBT and cELISA diagnostic tests and estimate

true disease prevalence for cattle, sheep and goats of northern Tanzania. Using this Bayesian latent class model, RBT and cELISA test outcome probabilities conditional on an unknown livestock disease status were specified using the sensitivity (Se) and specificity (Sp) of the two diagnostic tests and the prevalence (p) of disease in two subpopulations (Branscum et al., 2005; Mazeri et al., 2016). For this analysis, the RBT and cELISA test results were specified as either positive or negative, and diagnostic test data were classified as one of two subpopulations: exclusive pastoralist; or non-exclusive pastoralist. The model assumes that for the $i$th subpopulation, the counts ($O_i$) with each combination of test results (+/+; +/-; -/+; -/-) follows a multinomial distribution (Bronsvoort et al., 2009; Mazeri et al., 2016; Toft et al., 2005):

$$O_i|\ Se_j, Sp_j, p_i \sim \text{Multinomial}(\mathbf{Pr_i}, n_i) \quad \text{for } i = 1,2,\dots,S \quad \text{and } j = 1,2,\dots,T$$

Where $\mathbf{Pr_i}$ is a vector of probabilities of observing the 4 combinations of diagnostic test results for the $i$th subpopulation, $n_i$ is the total number of observations of the $i$th subpopulation, S is the number of different subpopulations and T is the number of diagnostic tests.

Individual test estimates for sensitivity and specificity were then used to estimate an overall sensitivity and specificity for in-series and in-parallel diagnostic testing approaches. The in-series diagnostic testing approach only identified animals as positive if the animal tested positive by both RBT and cELISA. The in-parallel approach identified an animal as positive if test positive by either RBT or cELISA. The equations for sensitivity and specificity estimates using in-series and in-parallel diagnostic testing approaches are as follows:

$$\text{Series Se} = Se_1 * Se_2$$
$$\text{Series Sp} = 1 - (1 - Sp_1) * (1 - Sp_2)$$
$$\text{Parallel Se} = 1 - (1 - Se_1) * (1 - Se_2)$$
$$\text{Parallel Sp} = Sp_1 * Sp_2$$

### 4.2.8 Statistical analyses: Model assumptions

Model assumption (a) was a difference in disease prevalence between the subpopulations being tested. The data for cattle, sheep and goats were split into

two subpopulations: exclusive pastoralist and non-exclusive pastoralist. It can be assumed that brucellosis prevalence is different between exclusive pastoralist communities and other livestock-keeping communities (Racloz et al., 2013), which has been reported in Tanzania (Shirima et al., 2010; Swai and Schoonman, 2010). Therefore, model assumption (a) was assumed to be met.

Model assumption (b) was that a diagnostic test performs comparably across subpopulations. This assumption was considered to be met because the majority of cattle, sheep and goat samples used in this analysis were of the same indigenous breed, the RBT and cELISA testing followed the same standard operating procedures, and test antigens and control sera were sourced from the same manufacturer.

The final model assumption (c) was that of conditional independence regarding disease status between RBT and cELISA tests. As RBT and cELISA are both serological tests that detect an antibody response to *Brucella* spp. exposure, it was likely that this model assumption could not be met. Therefore, the model was extended to include conditional dependence between diagnostic tests using a covariance parameterisation (Branscum et al., 2005; Bronsvoort et al., 2019; Mazeri et al., 2016):

$$\Pr(T_1+, T_2+) = \left((Se_1 * Se_2) + covDp\right) * p_i + \left(((1 - Sp_1) * (1 - Sp_2)) + covDn\right) * (1 - p_i)$$

$$\Pr(T_1+, T_2-) = \left(((Se_1) * (1 - Se_2)) - covDp\right) * p_i + \left(((1 - Sp_1) * Sp_2) - covDn\right) * (1 - p_i)$$

$$\Pr(T_1-, T_2+) = \left((1 - Se_1) * Se_2) - covDp\right) * p_i + \left((Sp_1 * (1 - Sp_2)) - covDn\right) * (1 - p_i)$$

$$\Pr(T_1-, T_2-) = \left((1 - Se_1) * (1 - Se_2) + covDp\right) * p_i + \left((Sp_1 * Sp_2) + covDn\right) * (1 - p_i)$$

Where Pr is the probability of observing the specific combination of diagnostic test outcomes (+/+; +/-; -/+; -/-), T+ is diagnostic test positive and T- is diagnostic test negative, $_1$ represents the RBT, $_2$ represents the cELISA. The covDp and covDn are the covariance between the diagnostic tests when an animal is disease positive or disease negative, respectively, and $p_i$ represents infection prevalence in the *i*th subpopulations.

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The inclusion of conditional dependence in a two-test, two-population model increases the number of parameters being estimated to eight, with only six degrees of freedom available. In order to allow model parameter estimation in this case, it was necessary that at least some of the model parameters had informative (non-uniform) priors (Branscum et al., 2005).

## 4.2.9 Statistical analyses: Prior distributions

For the cattle model, a set of test sensitivity and specificity prior distributions were informed by literature on RBT and cELISA test performance mean estimates in Africa (see Tables 4.1 and 4.2). For sheep and goats, a set of RBT and cELISA test parameter prior distributions were informed by the available literature reporting mean estimates for diagnostic test performance outside of Africa (see Tables 4.3 and 4.4).

The beta distribution shape parameters ($\alpha$, $\beta$) for test sensitivity and specificity priors used to analyse data from cattle were calculated by extracting the mean estimates from Tables 4.1 and 4.2. The 'fitdist' function found in the *fitdistrplus* R package (Delignette-Muller et al., 2019) was used to estimate the beta distribution shape parameters from these data. The same approach was applied to generate priors for the analysis of both sheep and goat data using the mean estimates of RBT and cELISA specificity and sensitivity found in Table 4.3 and 4.4. Beta distribution parameters to the nearest integer for cattle and small ruminants are given in Table 4.5.

**Table 4.1: Sensitivity (Se) and specificity (Sp) mean estimates using the Rose Bengal plate test (RBT) for cattle in Africa**, *LCA is latent class analysis*

| Reference | Test property estimation technique | Location | Se | Sp |
|---|---|---|---|---|
| (Muma et al., 2007) | LCA uniform priors | Zambia | 0.930 | 0.820 |
| (Matope et al., 2011) | LCA uniform priors | Zimbabwe | 0.947 | 0.990 |
| (Sanogo et al., 2013) | LCA informed priors: (Nielsen, 2002) | Ivory Coast | 0.547 | 0.978 |
| (Etman et al., 2014) | Culture & vaccinated | Egypt | 0.961 | 0.993 |
| (Getachew et al., 2016) | LCA informed priors: (Gall and Nielsen, 2004; Mainar-Jaime et al., 2005) | Ethiopia | 0.896 | 0.845 |
| (Chisi et al., 2017) | Culture | South Africa | 0.958 | 1 |
| (Hosein et al., 2017) | Culture | Egypt | 0.943 | 0.857 |

**Table 4.2: Sensitivity (Se) and specificity (Sp) mean estimates using the competitive enzyme-linked immunosorbent assay (cELISA) for cattle in Africa**, *LCA is latent class analysis*

| Reference | Test property estimation technique | Location | Se | Sp |
|---|---|---|---|---|
| (Muma et al., 2007) | LCA uniform priors | Zambia | 0.970 | 0.600 |
| (Bronsvoort et al., 2009) | LCA informed priors: (Fosgate et al., 2003; Gall et al., 1998; McGiven et al., 2003; Nielsen et al., 1996, 1995; Stack et al., 1999) | Cameroon | 0.978 | 0.987 |
| (Matope et al., 2011) | LCA uniform priors | Zimbabwe | 0.990 | 0.954 |
| (Etman et al., 2014) | Culture | Egypt | 0.971 | 1 |
| (Chisi et al., 2017) | Culture | South Africa | 0.939 | 0.950 |

**Table 4.3: Sensitivity (Se) and specificity (Sp) mean estimates using the Rose Bengal plate test (RBT) for small ruminants,** *S is sheep, G is goats and SG is sheep and goats, LCA is latent class analysis*

| Reference | Test property estimation technique | Location | Species | Se | Sp |
|---|---|---|---|---|---|
| (Marín et al., 1999) | Culture | Unknown | S | 1 | 1 |
| (Nielsen et al., 2004) | Experimentally infected | Canada & Unknown | S | 0.997 | 0.880 |
| (Minas et al., 2005) | Culture | Greece | S | 0.904 | 0.996 |
| (EFSA, 2006) | Culture | Multiple | SG | 0.925 | 0.999 |
| (Minas et al., 2008) | Culture | Greece | SG | 0.758 | 0.997 |
| (Ramírez-Pfeiffer et al., 2008) | Culture | Mexico & Canada | G | 0.997 | 0.327 |
| (Rahman et al., 2013) | LCA informed priors: (Abuharfeil and Abo-Shehada, 1998; Blasco et al., 1994; EFSA, 2006; Minas et al., 2008, 2005; Nielsen et al., 2004; Ramírez-Pfeiffer et al., 2008) | Bangladesh | S<br>G | 0.828<br>0.802 | 0.983<br>0.996 |
| (García-Bocanegra et al., 2014) | LCA informed priors: (EFSA, 2006) | Spain | S | 0.750 | 0.974 |
| (Gupta et al., 2014) | Culture | India | G | 0.700 | 0.900 |

**Table 4.4: Sensitivity (Se) and specificity (Sp) mean estimates of the competitive enzyme-linked immunosorbent assay (cELISA) for small ruminants,** *S is sheep, G is goats and SG is sheep and goats*

| Reference | Test property estimation technique | Location | Species | Se | Sp |
|---|---|---|---|---|---|
| (Marín et al., 1999) | Culture | Unknown | S | 0.960 | 1 |
| (Burriel et al., 2004) | Vaccinated | Greece | SG | 0.885 | 0.985 |
| (Nielsen et al., 2004) | Experimentally infected | Canada & Unknown | S | 0.750 | 0.998 |
| (Minas et al., 2005) | Culture | Greece | S | 0.964 | 0.994 |
| (Nielsen et al., 2005) | Culture | Mexico & Canada | G | 0.936 | 0.994 |
| (Minas et al., 2008) | Culture | Greece | SG | 0.779 | 0.972 |
| (García-Bocanegra et al., 2014) | Culture | Spain | S | 1 | 1 |

**Table 4.5: Prior distribution diagnostic test parameter estimates for sensitivity and specificity of the Rose Bengal plate test (RBT) and the competitive enzyme-linked immunosorbent assay (cELISA) used for cattle, sheep and goat models**, *Se is sensitivity and Sp is specificity*

| Test parameters | | Beta distribution ($\alpha$, $\beta$) | |
|---|---|---|---|
| | | Cattle | Small ruminants |
| RBT | Se | (8, 1) | (4, 1) |
| | Sp | (7, 1) | (2, 1) |
| cELISA | Se | (102, 3) | (7, 1) |
| | Sp | (4, 1) | (104, 1) |

Brucellosis prevalence priors for exclusive pastoralist and non-exclusive pastoralist subpopulations were informed by other published studies in Tanzania, that estimated brucellosis seroprevalence using RBT and or cELISA tests in similar livestock-keeping systems with a comparable study design (see Table 4.6 for cattle and Table 4.7 for sheep and goats). A vague prevalence prior of 0 to 0.49 was set for all livestock models, which encompassed the range of seroprevalence estimates in the literature and allowed exploration of the wider parameter space.

**Table 4.6: Brucellosis seroprevalence estimates for cattle in Tanzania generated using the Rose Bengal plate test (RBT) and/or the competitive enzyme-linked immunosorbent assay (cELISA)**

| Prevalence (%) | Study design | Livestock-keeping system | Tests | Location | Reference |
|---|---|---|---|---|---|
| 3.0 | Matched case-control | Pastoral/ Agro-pastoral | RBT & cELISA | Arusha & Manyara | (John et al., 2010) |
| 4.1 | Cross-sectional | Smallholder | RBT | Tanga | (Swai and Schoonman, 2010) |
| 4.9 | Cross-sectional | Pastoral/ Agro-pastoral | cELISA | Arusha & Manyara | (Shirima et al., 2010) |
| 5.6 | Cross-sectional | Agro-pastoral | RBT & cELISA | Kigoma | (Chitupila et al., 2015) |
| 5.6 | Cross-sectional | Agro-pastoral | RBT & cELISA | Mara | (Shirima and Kunda, 2016) |
| 6.8 | Cross-sectional | Agro-pastoral | RBT & cELISA | Katavi | (Assenga et al., 2015) |
| 7.3 | Cross-sectional | Pastoral | RBT | Tanga | (Swai and Schoonman, 2010) |
| 9.3 | Cross-sectional | Agro-pastoral/ Commercial | RBT & cELISA | Mbeya | (Sagamiko et al., 2018) |
| 10.0 | Cross-sectional | Pastoral | RBT | Arusha | (Mellau et al., 2009) |
| 18.4 | Cross-sectional | Smallholder | cELISA | Morogoro | (Lyimo, 2013) |
| 21.5 | Cross-sectional | Commercial | RBT | Mbeya | (Mathew et al., 2015) |

**Table 4.7: Brucellosis seroprevalence estimates for sheep and goats in Tanzania using the Rose Bengal plate test (RBT) and/or the competitive enzyme-linked immunosorbent assay (cELISA),** *S is sheep, G is goats and SG is sheep and goats*

| Prevalence (%) | Species | Study design | Livestock-keeping system | Tests | Location | Reference |
|---|---|---|---|---|---|---|
| 0<br>0 | S<br>G | Cross-sectional | Commercial | RBT | Mbeya | (Mathew et al., 2015) |
| 0 | SG | Cross-sectional | Agro-pastoral | RBT & cELISA | Mara | (Shirima and Kunda, 2016) |
| 1.6 | G | Cross-sectional | Agro-pastoral | RBT & cELISA | Katavi | (Assenga et al., 2015) |
| 3.4<br>4.6 | S<br>G | Matched case-control | Pastoral/ Agro-pastoral | RBT & cELISA | Arusha & Manyara | (John et al., 2010) |
| 6.5 | SG | Cross-sectional | Pastoral/ Agro-pastoral | cELISA | Arusha & Manyara | (Shirima et al., 2010) |
| 7.7<br>11.5 | S<br>G | Cross-sectional | Pastoral | RBT | Arusha | (Mellau et al., 2009) |

## 4.2.10 Statistical analyses: Model implementation

Three species models including: a bovine model (using cattle data with cattle prior distributions); an ovine model (using sheep data with small ruminant prior distributions); and a caprine model (using goat data with small ruminant prior distributions) were implemented separately. All livestock models were implemented with JAGS (Plummer, 2003) in R software version 3.6.1 (R Core Team, 2019), using the *rjags* R package (Plummer et al., 2019b). Three Markov Chain Monte Carlo (MCMC) chains with different initial starting values were used. The first 50,000 iterations were considered burn-in and discarded. Another 250,000 iterations were run per chain. Of these, every 100$^{th}$ iteration per chain, totalling 7,500 iterations, was used to inform the posterior distribution. Model posterior distributions included the mean estimate and associated 0.025 and 0.975 Bayesian credibility intervals (BCI).

## 4.2.11 Statistical analyses: Model sensitivity analyses

Sensitivity analyses were performed to explore the effects of relaxing the literature-informed prior distributions on posterior inference. Each livestock species model was implemented with a series of three test parameter prior sets including: the 'strict' literature-informed priors described above; uniformly distributed priors; and a 'relaxed' prior set distribution between the strict and uniform priors. The beta distributions for each prior set in cattle, sheep and goat models are given in Table 4.8, 4.9 and 4.10. Frequency plots of prior and posterior distributions were visually inspected to select a prior set that looked to avoid inversion of the parameter space or overwhelming the data by driving the model posterior estimates.

**Table 4.8: Beta distributions (dbeta) used to define priors for sensitivity (Se) and specificity (Sp) of Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) parameters for the bovine model,** *ranging from uniform prior distributions to literature-informed strict prior distributions*

| Parameter | dbeta($\alpha$,$\beta$) | | |
|---|---|---|---|
| | Uniform priors | Relaxed priors | Strict priors |
| Se RBT | 1,1 | 7,1 | 8,1 |
| Sp RBT | 1,1 | 1,1 | 7,1 |
| Se cELISA | 1,1 | 30,2 | 102,3 |
| Sp cELISA | 1,1 | 1,1 | 4,1 |

**Table 4.9: Beta distributions (dbeta) used to define priors for sensitivity (Se) and specificity (Sp) of Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) parameters for the ovine model,** *ranging from uniform prior distributions to literature-informed strict prior distributions*

| Parameter | dbeta($\alpha$,$\beta$) | | |
|---|---|---|---|
| | Uniform priors | Relaxed priors | Strict priors |
| Se RBT | 1,1 | 4,1 | 4,1 |
| Sp RBT | 1,1 | 1,1 | 2,1 |
| Se cELISA | 1,1 | 7,1 | 7,1 |
| Sp cELISA | 1,1 | 1,1 | 104,1 |

**Table 4.10: Beta distributions (dbeta) used to define priors for sensitivity (Se) and specificity (Sp) of Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) parameters for the caprine model,** *ranging from uniform prior distributions to literature-informed strict prior distributions*

| Parameter | dbeta($\alpha$,$\beta$) | | |
|---|---|---|---|
| | Uniform priors | Relaxed priors | Strict priors |
| Se RBT | 1,1 | 4,1 | 4,1 |
| Sp RBT | 1,1 | 1,1 | 2,1 |
| Se cELISA | 1,1 | 7,1 | 7,1 |
| Sp cELISA | 1,1 | 1,1 | 104,1 |

## 4.2.12 Statistical analyses: Model diagnostics

All models were implemented with and without the covariance parameterisation, so that the effect of assuming conditional dependence (with covariance) between RBT and cELISA could be evaluated. Model selection was performed by comparing posterior estimates and deviance information criterion (DIC) scores, which is a Bayesian measure of model fit (Spiegelhalter et al., 2002).

MCMC chain convergence was assessed using the Gelman-Rubin potential scale reduction factor and by visual inspection of Gelman-Rubin, density and trace plots for each parameter of the model to confirm satisfactory convergence. Model diagnostics and visualisation were performed using the *coda* R package (Plummer et al., 2019a).

## 4.2.13 Statistical analyses: Positive and negative predictive values

The positive predictive value (PPV) and negative predictive value (NPV) were calculated for four diagnostic testing approaches in the pastoralist subpopulation for each final model. The diagnostic test approaches included: RBT; cELISA; in-series; and in-parallel. PPV and NPV measure the probability that when a test is positive that the animal actually has the disease and that when a test is negative that the animal really does not have the disease, respectively (Dohoo et al., 2003b). PPV and NPV are based on Bayes' theorem (Price and Bayes, 1763), and are estimated using the posterior estimates for the true subpopulation prevalence (p), sensitivity (Se) and specificity (Sp) (Linn, 2004):

$$PPV = \frac{Se * p}{(Se * p) + (1 - Sp) * (1 - p)}$$

$$NPV = \frac{Sp * (1 - p)}{\left(Sp * (1 - p)\right) + (1 - Se) * p}$$

The final model RBT, cELISA, in-series and in-parallel sensitivity and specificity estimates and PPVs and NPVs were compared. The diagnostic testing approach

with a high combined sensitivity and specificity as well as high NPV was considered the most effective approach.

## 4.3 Results

### 4.3.1 Livestock data

Data available for the bovine model included 3479 cattle samples both RBT and cELISA tested, of which 2064 were from the exclusive pastoralist livestock-keeping subpopulation and 1415 from the non-exclusive pastoralist livestock-keeping subpopulation. Ovine data included a total of 2516 samples, 1739 from the exclusive pastoralist subpopulation and 777 from the non-exclusive pastoralist subpopulation. A total of 3193 samples were available for the caprine model, 1892 samples were from the exclusive pastoralist subpopulation and 1301 from the non-exclusive pastoralist subpopulation. The total number of RBT and cELISA test positives for each livestock species in exclusive and non-exclusive pastoralist subpopulations are given in Table 4.11.

**Table 4.11: Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) positive test results for cattle, sheep and goats in exclusive pastoralist and non-exclusive pastoralist subpopulations from two cross-sectional studies in northern Tanzania,** *N is total number of samples tested, n is total number of test positive samples*

| Livestock species | Exclusive pastoralist subpopulation | | Non-exclusive pastoralist subpopulation | |
|---|---|---|---|---|
| | RBT n/N | cELISA n/N | RBT n/N | cELISA n/N |
| Cattle | 89/2064 | 120/2064 | 12/1415 | 21/1415 |
| Sheep | 36/1739 | 59/1739 | 15/777 | 5/777 |
| Goat | 81/1892 | 96/1892 | 25/1301 | 19/1301 |

### 4.3.2 Bovine final model: Specifications

Sensitivity, specificity and prevalence estimates for each diagnostic test generated by running the bovine model with and without a covariance parameterisation and using three prior sets are compared in Figure 4.2. The posterior mean estimates and 95% BCI for in-series and in-parallel diagnostic testing approaches generated by running the bovine model with and without a covariance parameterisation and using three prior sets are given in Figure 4.3. Frequency plots were also visually inspected for the bovine model using the three prior sets (see Appendix 4 for frequency plots). All model runs showed satisfactory convergence (see Appendix 4 for diagnostic plots). Relaxed priors were selected for the final model. The DIC value for a model with and without

the covariance parameterisation did not indicate a major difference (≥2 points) between the two models, so the simpler model was selected (see Table 4.12). Therefore, the bovine model assuming conditional independence (without covariance) and the relaxed prior set was selected as the final model for the analysis of cattle data.
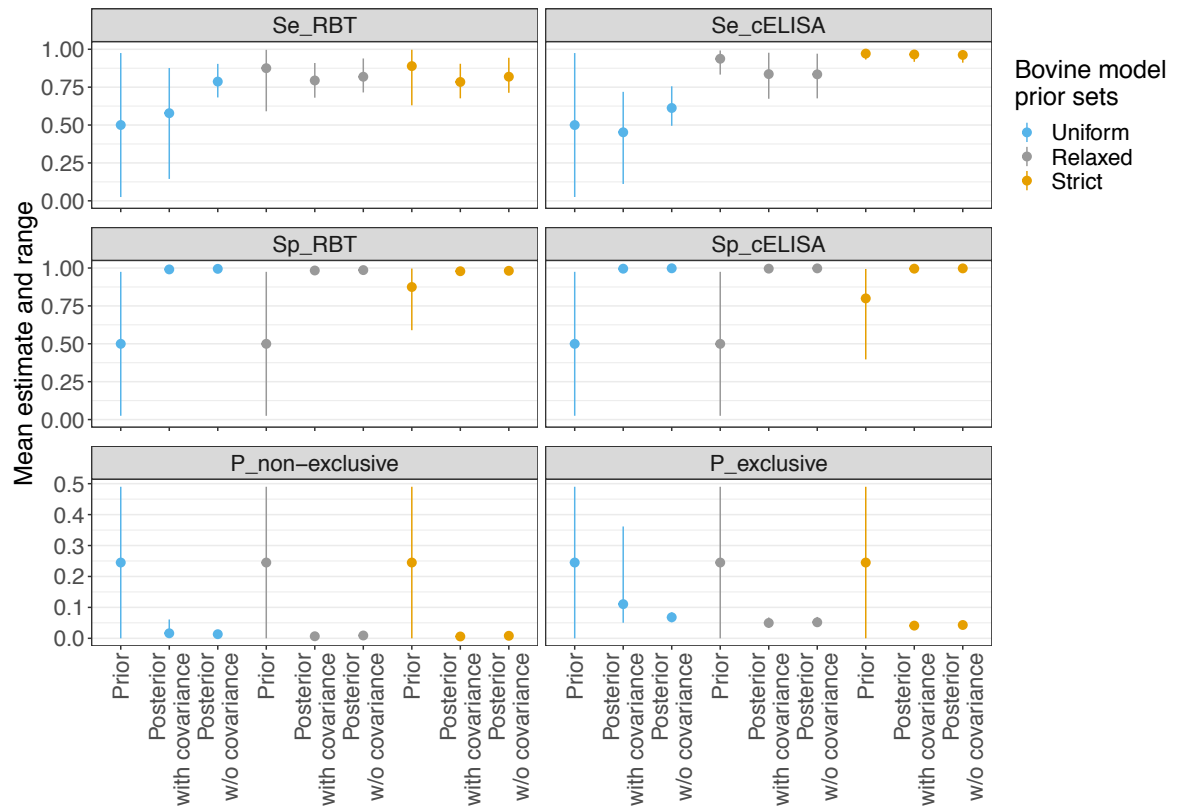


**Figure 4.2: Sensitivity, specificity and prevalence mean estimates and ranges for each combination of bovine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)**, *w/o denotes without, Se is sensitivity, Sp is specificity, RBT is Rose Bengal plate test, cELISA is competitive enzyme-linked immunosorbent assay, P is prevalence, non-exclusive is the non-exclusive pastoralist subpopulation and exclusive is the exclusive pastoralist subpopulation*
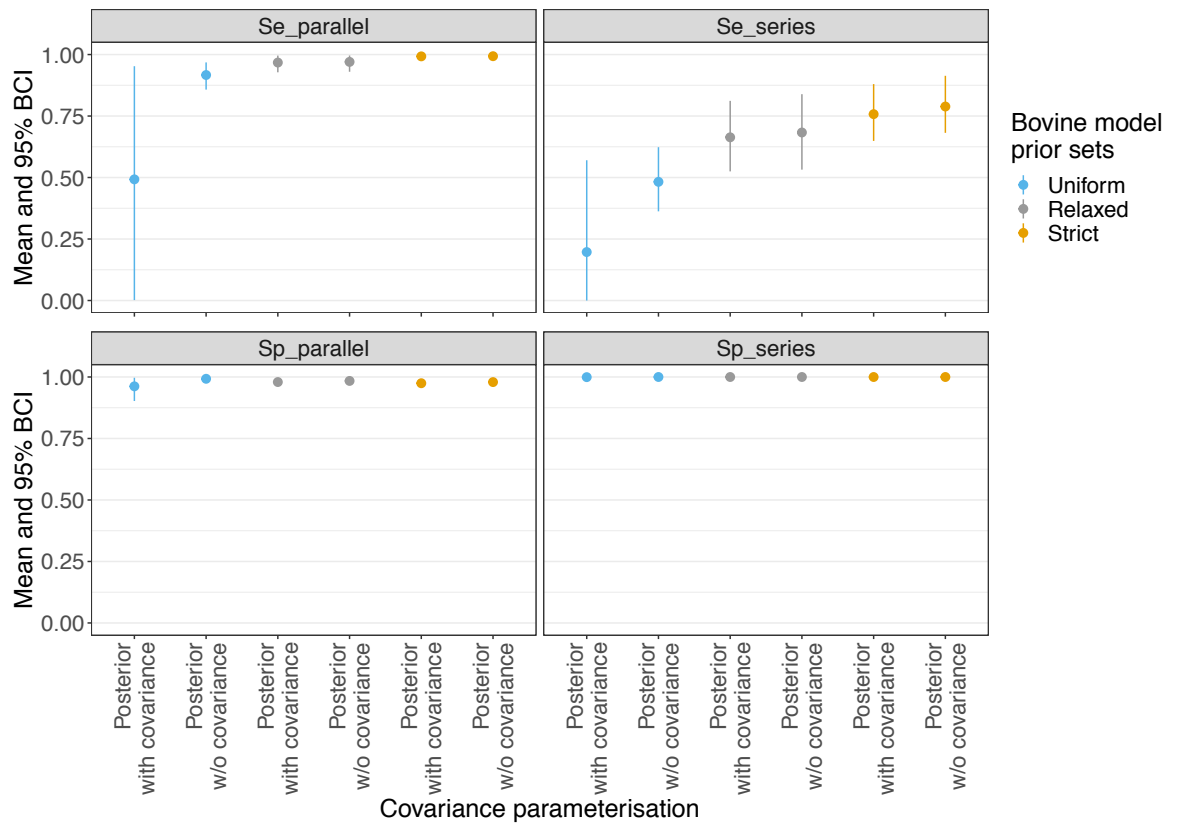
**Figure 4.3: In-series and in-parallel sensitivity and specificity mean posterior estimates and 95% Bayesian credibility intervals (BCI) for each combination of bovine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)**, *w/o denotes without, Se_parallel is in-parallel sensitivity, Se_series is in-series sensitivity, Sp_parallel is in-parallel specificity, Sp_series is in-series specificity*

**Table 4.12: Deviance information criterion (DIC) scores for each combination of bovine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)**

| Model assumption | DIC | | |
|---|---|---|---|
| | Uniform priors | Relaxed priors | Strict priors |
| With covariance | 39.37 | 46.62 | 52.44 |
| Without covariance | 39.27 | 47.10 | 52.76 |

### 4.3.3 Bovine final model: Estimates and diagnostics

The final bovine model mean posterior and 95% BCI estimates for sensitivity and specificity of the RBT, cELISA, in-series and in-parallel model parameters as well as prevalence in exclusive and non-exclusive pastoralist subpopulations are given in Table 4.13. The mean posterior sensitivity for the RBT and cELISA tests were estimated as 0.819 (95% BCI: 0.715-0.939) and 0.835 (95% BCI: 0.678-0.971), respectively. The mean posterior estimates for specificity of the RBT and cELISA were 0.986 (95% BCI: 0.979-0.994) and 0.998 (95% BCI: 0.993-1), respectively. The estimated prevalence of bovine brucellosis in the exclusive pastoralist subpopulation was 0.052 (95% BCI: 0.037-0.069) and in the non-exclusive pastoralist subpopulation was 0.009 (95% BCI: 0.004-0.016). The final bovine model had a Gelman-Rubin reduction factor of <1.1 and showed satisfactory convergence for all model parameters (see Figure 4.4, 4.5 and 4.6). The PPV and NPV exclusive pastoralist subpopulation estimates for the final bovine model are given in Table 4.14. The best diagnostic testing approach for the final bovine model was in-parallel. Sensitivity and specificity in-parallel were estimated at 0.970 (95% BCI: 0.930-0.996) and 0.984 (95% BCI: 0.976-0.992), respectively (see Table 4.13). For the model code for the final bovine model see Appendix 5.

**Table 4.13: Mean posterior parameter estimates and 95% Bayesian Credibility Intervals (BCI) for the final bovine model using the relaxed prior set\* and assuming conditional independence,** *Se is sensitivity, Sp is specificity, RBT is Rose Bengal plate test and cELISA is competitive enzyme-linked immunosorbent assay*

| Parameter | Mean | 2.5% BCI | 97.5% BCI |
| --- | --- | --- | --- |
| Se: RBT | 0.819 | 0.715 | 0.939 |
| Sp: RBT | 0.986 | 0.979 | 0.994 |
| Se: cELISA | 0.835 | 0.678 | 0.971 |
| Sp: cELISA | 0.998 | 0.993 | 1 |
| Se: in-series | 0.683 | 0.533 | 0.840 |
| Sp: in-series | 1 | 1 | 1 |
| Se: in-parallel | 0.970 | 0.930 | 0.996 |
| Sp: in-parallel | 0.984 | 0.976 | 0.992 |
| Prevalence: exclusive pastoralist subpopulation | 0.052 | 0.037 | 0.069 |
| Prevalence: non-exclusive pastoralist subpopulation | 0.009 | 0.004 | 0.016 |

\*relaxed prior set:
Se RBT ~ dbeta(7,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(30,2)
Sp cELISA ~ dbeta(1,1)

**Table 4.14: Positive and negative predictive values for RBT, cELISA, in-series and in-parallel diagnostic approaches in an exclusive pastoralist subpopulation for the final bovine model**, *RBT is Rose Bengal plate test and cELISA is competitive enzyme-linked immunosorbent assay, in-series is testing all RBT positive samples by cELISA, in-parallel is testing all samples by RBT and cELISA*

| Diagnostic approach | Positive Predictive Value | Negative Predictive Value |
|---|---|---|
| RBT | 0.762 | 0.990 |
| cELISA | 0.958 | 0.991 |
| In-series | 1 | 0.983 |
| In-parallel | 0.769 | 0.998 |
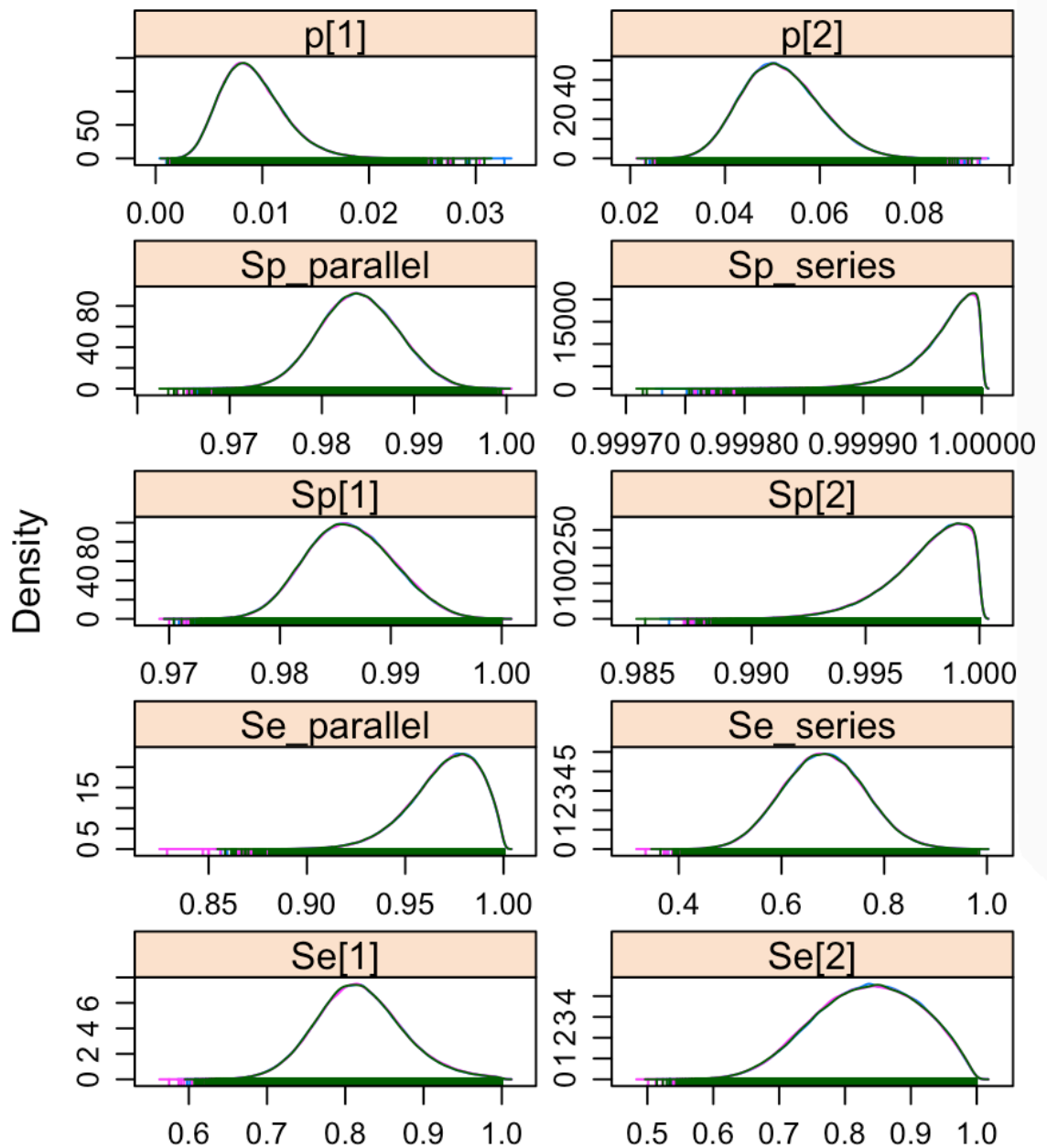
**Figure 4.4: Density plots for each parameter of the final bovine model**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist subpopulation and p[2] is prevalence in an exclusive pastoralist subpopulation*
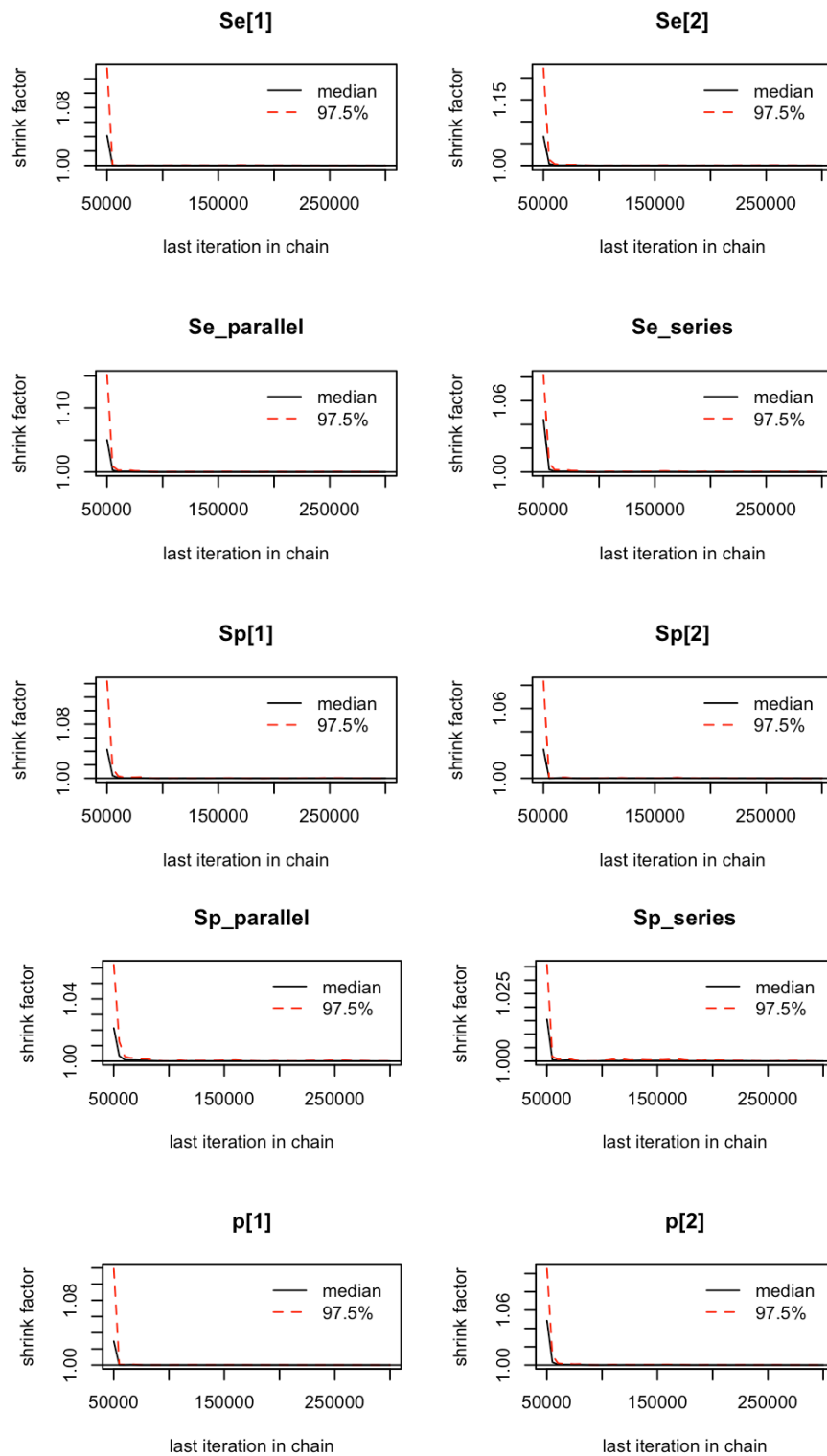
**Figure 4.5: Gelman-Rubin diagnostic plots showing convergence for each parameter of the final bovine model**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist subpopulation and p[2] is prevalence in an exclusive pastoralist subpopulation*

**Figure 4.6: Trace plots showing MCMC chain convergence for each parameter of the final bovine model**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist subpopulation and p[2] is prevalence in an exclusive pastoralist subpopulation*

## 4.3.4 Ovine final model: Specifications

Sensitivity, specificity and prevalence estimates for each diagnostic test generated by running the ovine model with and without a covariance parameterisation and using three prior sets are compared in Figure 4.7. The posterior mean estimates and 95% BCI for in-series and in-parallel diagnostic testing approaches generated by running the ovine model with and without a covariance parameterisation and using three prior sets are given in Figure 4.8.
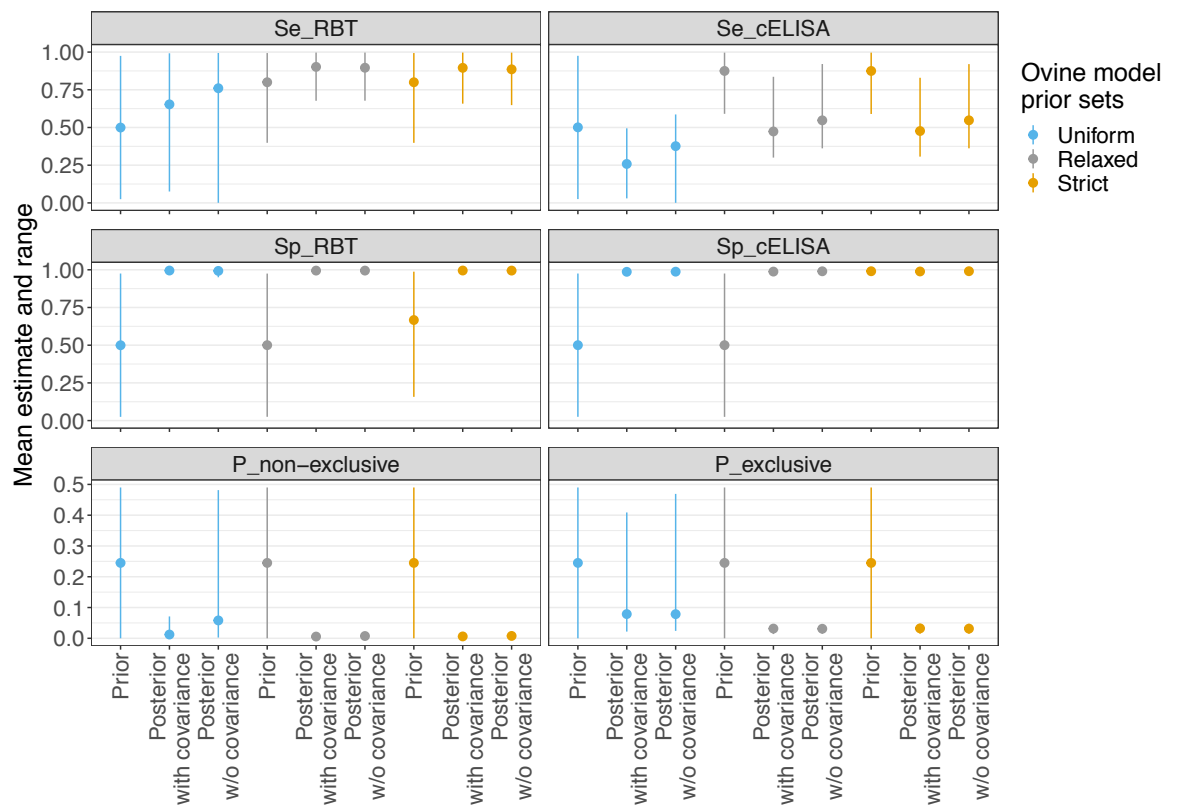


**Figure 4.7: Sensitivity, specificity and prevalence mean estimates and ranges for each combination of ovine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)**, *w/o denotes without, Se is sensitivity, Sp is specificity, RBT is Rose Bengal plate test, cELISA is competitive enzyme-linked immunosorbent assay, P is prevalence, non-exclusive is the non-exclusive pastoralist subpopulation and exclusive is the exclusive pastoralist subpopulation*
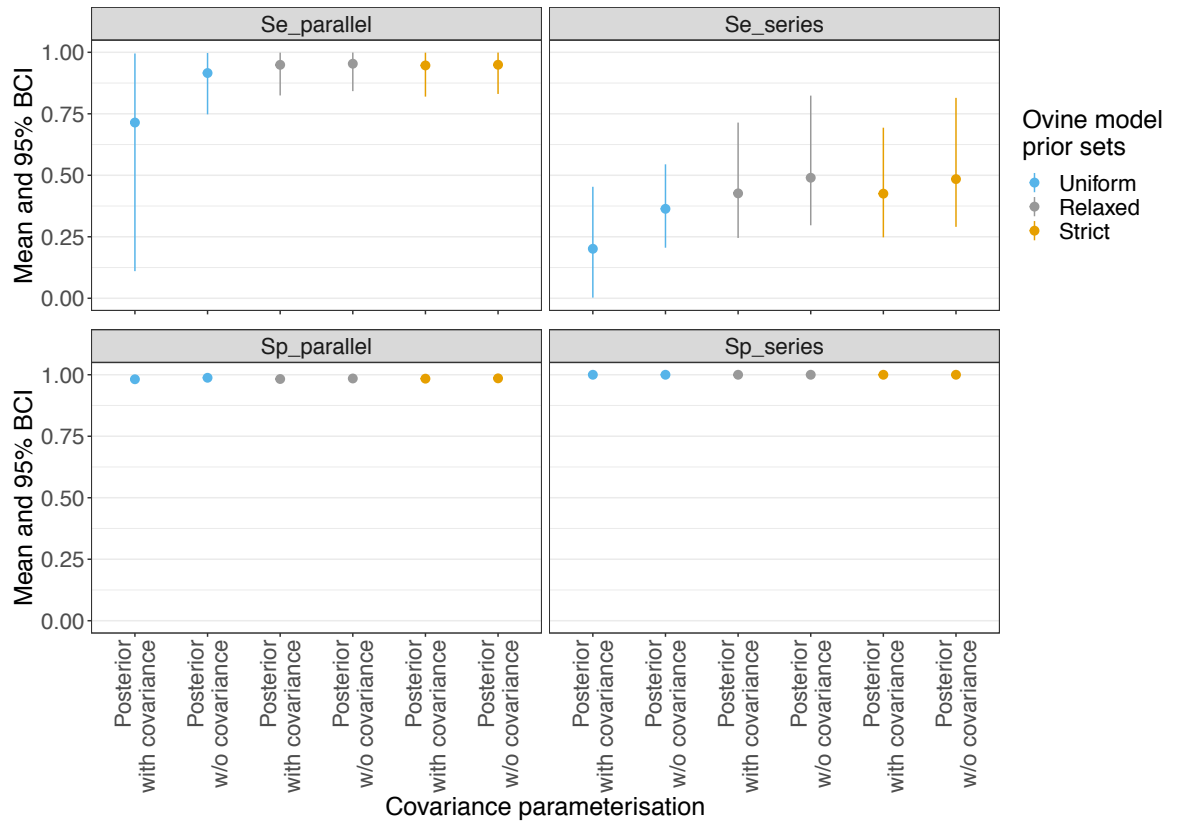
**Figure 4.8: In-series and in-parallel sensitivity and specificity mean posterior estimates and 95% Bayesian credibility intervals (BCI) for each combination of ovine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)***, w/o denotes without, Se_parallel is in-parallel sensitivity, Se_series is in-series sensitivity, Sp_parallel is in-parallel specificity, Sp_series is in-series specificity*

Frequency plots were also visually inspected for the ovine model using the three prior sets (see Appendix 6 for frequency plots). All model runs showed satisfactory convergence (see Appendix 6 for diagnostic plots). Relaxed priors were selected for the final model. The ovine model with covariance gave a lower DIC value (<2 points) than the model without covariance, so the more complex model was selected (see Table 4.15). Therefore, the ovine model assuming conditional dependence (with covariance) and the relaxed prior set was selected as the final ovine model for the analysis of sheep data.

**Table 4.15: Deviance information criterion (DIC) scores for each combination of ovine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)**

| Model assumption | DIC | | |
|---|---|---|---|
| | Uniform priors | Relaxed priors | Strict priors |
| With covariance | 38.31 | 40.63 | 40.76 |
| Without covariance | 40.16 | 42.95 | 43.19 |

### 4.3.5 Ovine final model: Estimates and diagnostics

The ovine final model mean posterior and 95% BCI estimates for sensitivity and specificity of the RBT, cELISA, in-series and in-parallel model parameters as well as prevalence in exclusive and non-exclusive pastoralist subpopulations are shown in Table 4.16. The mean posterior sensitivity for the RBT and cELISA tests were estimated as 0.902 (95% BCI: 0.679-0.997) and 0.472 (95% BCI: 0.301-0.821), respectively. The mean posterior estimates for specificity of the RBT and cELISA were 0.995 (95% BCI: 0.983-1) and 0.988 (95% BCI: 0.982-0.993), respectively. The estimated prevalence of ovine brucellosis in the exclusive pastoralist subpopulation was 0.032 (95% BCI: 0.014-0.046) and in the non-exclusive pastoralist subpopulation was 0.006 (95% BCI: 0.000-0.015). The ovine final model had a Gelman-Rubin reduction factor of <1.1 and showed satisfactory convergence for all model parameters (see Appendix 7 for ovine final model diagnostic plots). The PPV and NPV exclusive pastoralist subpopulation estimates for the final ovine model are given in Table 4.17. The best diagnostic testing approach for the final ovine model was in-parallel. Sensitivity and specificity in-parallel were estimated at 0.949 (95% BCI: 0.823-0.999) and 0.983 (95% BCI: 0.969-0.992), respectively (see Table 4.16). The model code for the final ovine model is available in Appendix 8.

**Table 4.16: Mean posterior parameter estimates and 95% Bayesian Credibility Intervals (BCI) for the final ovine model using the relaxed prior set\* and assuming conditional dependence**, *Se is sensitivity, Sp is specificity, RBT is Rose Bengal plate test and cELISA is competitive enzyme-linked immunosorbent assay, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive*

| Parameter | Mean | 2.5% BCI | 97.5% BCI |
|---|---|---|---|
| Se: RBT | 0.902 | 0.679 | 0.997 |
| Sp: RBT | 0.995 | 0.983 | 1 |
| Se: cELISA | 0.472 | 0.301 | 0.821 |
| Sp: cELISA | 0.988 | 0.982 | 0.993 |
| Se: in-series | 0.425 | 0.245 | 0.705 |
| Sp: in-series | 1 | 1 | 1 |
| Se: in-parallel | 0.949 | 0.823 | 0.999 |
| Sp: in-parallel | 0.983 | 0.969 | 0.992 |
| Prevalence: exclusive pastoralist subpopulation | 0.032 | 0.014 | 0.046 |
| Prevalence: non-exclusive pastoralist subpopulation | 0.006 | 0.000 | 0.015 |
| covDn | 0.002 | 0.000 | 0.006 |
| covDp | -0.001 | -0.065 | 0.066 |

\*relaxed prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(1,1)

**Table 4.17: Positive and negative predictive values for RBT, cELISA, in-series and in-parallel diagnostic approaches in an exclusive pastoralist subpopulation for the final ovine model**, *RBT is Rose Bengal plate test and cELISA is competitive enzyme-linked immunosorbent assay, in-series is testing all RBT positive samples by cELISA, in-parallel is testing all samples by RBT and cELISA*

| Diagnostic approach | Positive Predictive Value | Negative Predictive Value |
|---|---|---|
| RBT | 0.856 | 0.997 |
| cELISA | 0.565 | 0.983 |
| In-series | 1 | 0.981 |
| In-parallel | 0.649 | 0.998 |

## 4.3.6 Caprine final model: Specifications

Sensitivity, specificity and prevalence estimates for each diagnostic test generated by running the caprine model with and without a covariance parameterisation and using three prior sets are compared in Figure 4.9. The posterior mean estimates and 95% BCI for in-series and in-parallel diagnostic testing approaches generated by running the caprine model with and without a covariance parameterisation and using three prior sets are given in Figure 4.10.
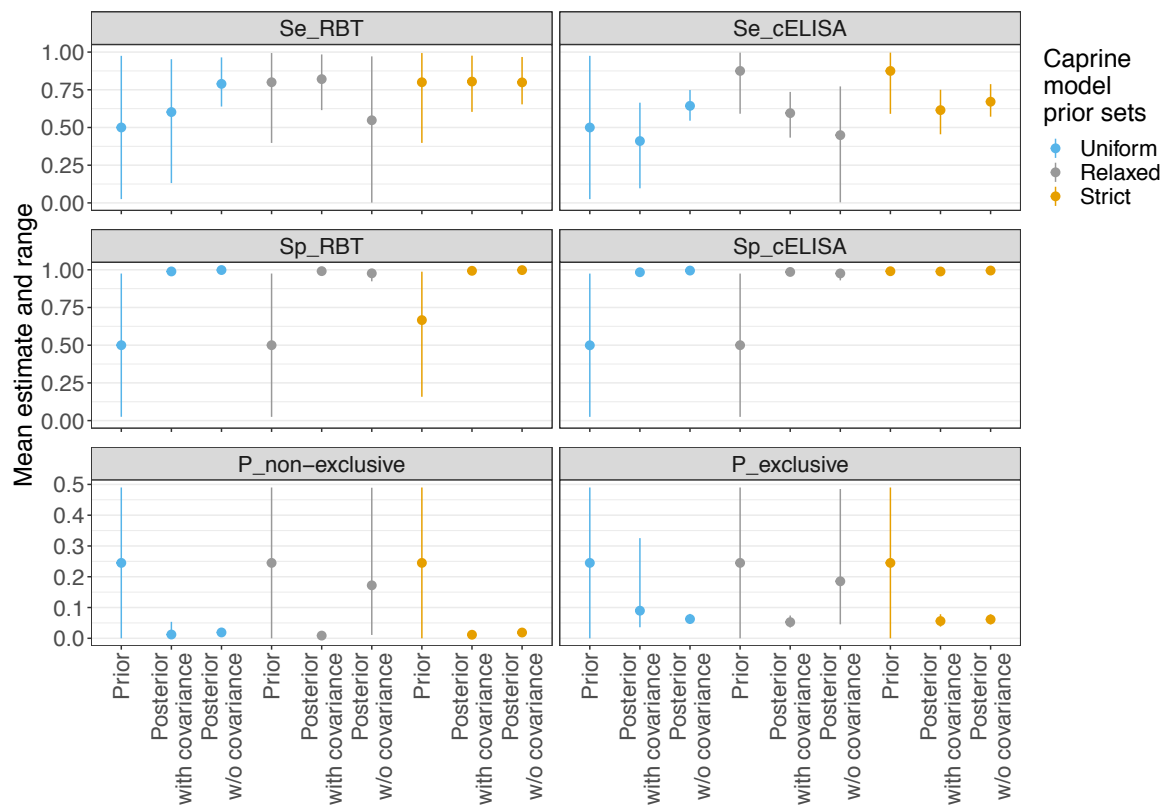


**Figure 4.9: Sensitivity, specificity and prevalence mean estimates and ranges for each combination of caprine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)**, *w/o denotes without, Se is sensitivity, Sp is specificity, RBT is Rose Bengal plate test, cELISA is competitive enzyme-linked immunosorbent assay, P is prevalence, non-exclusive is the non-exclusive pastoralist subpopulation and exclusive is the exclusive pastoralist subpopulation*
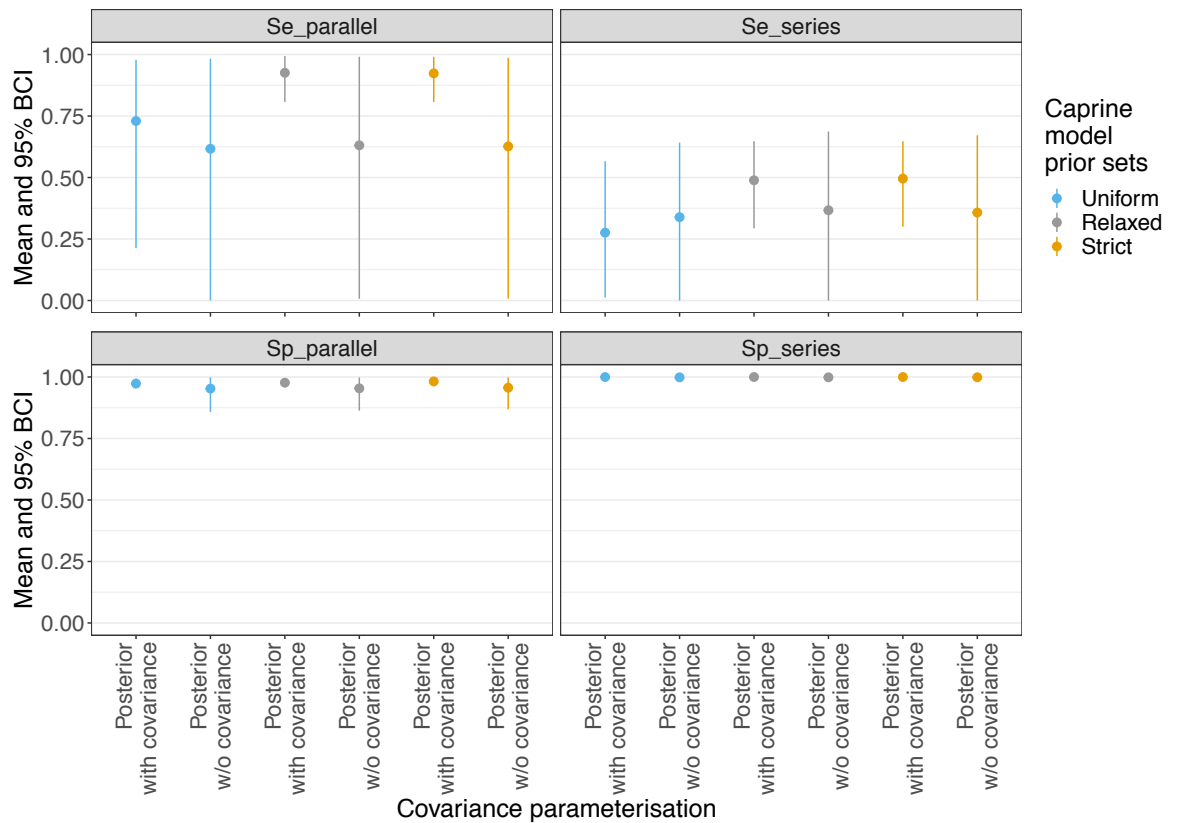
**Figure 4.10: In-series and in-parallel sensitivity and specificity mean posterior estimates and 95% Bayesian credibility intervals (BCI) for each combination of caprine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)**, *w/o denotes without, Se_parallel is in-parallel sensitivity, Se_series is in-series sensitivity, Sp_parallel is in-parallel specificity, Sp_series is in-series specificity*

Frequency plots were also visually inspected for the caprine model using the three prior sets (see Appendix 9 for frequency plots). All model runs showed satisfactory convergence (see Appendix 9 for diagnostic plots). Relaxed priors were selected for the final model. The caprine model with covariance gave a lower DIC value (<2 points) than the model not including the covariance parameterisation, so the more complex model was selected (see Table 4.18). Therefore, the caprine model assuming conditional dependence (with covariance) and the relaxed prior set was selected as the final caprine model for the analysis of goat data.

**Table 4.18: Deviance information criterion (DIC) scores for each combination of caprine model with uniform, relaxed and strict literature-informed prior sets, and conditional dependence (with covariance) or conditional independence (without covariance)**

| Model assumption | DIC | | |
|---|---|---|---|
| | Uniform priors | Relaxed priors | Strict priors |
| With covariance | 40.93 | 41.70 | 42.29 |
| Without covariance | 44.39 | 45.10 | 44.91 |

## 4.3.7 Caprine final model: Estimates and diagnostics

The caprine final model mean posterior and 95% BCI estimates for sensitivity and specificity of the RBT, cELISA, in-series and in-parallel model parameters as well as prevalence in exclusive and non-exclusive pastoralist subpopulations are shown in Table 4.19. The mean posterior sensitivity for the RBT and cELISA tests were estimated as 0.820 (95% BCI: 0.612-0.984) and 0.595 (95% BCI: 0.432-0.736), respectively. The mean posterior estimates for specificity of the RBT and cELISA were 0.991 (95% BCI: 0.981-1) and 0.986 (95% BCI: 0.976-0.997), respectively. The estimated prevalence of caprine brucellosis in the exclusive pastoralist subpopulation was 0.053 (95% BCI: 0.035-0.075) and in the non-exclusive pastoralist subpopulation was 0.009 (95% BCI: 0.000-0.023). The caprine final model had a Gelman-Rubin reduction factor of <1.1 and showed satisfactory convergence for all model parameters (see Appendix 10 for caprine final model diagnostic plots). The PPV and NPV exclusive pastoralist subpopulation estimates for the final caprine model are given in Table 4.20. The best diagnostic testing approach for the final caprine model was in-parallel. Sensitivity and specificity in-parallel were estimated at 0.926 (95% BCI: 0.807-0.994) and 0.977 (95% BCI: 0.959-0.995), respectively (see Table 4.19). The model code for the final caprine model is available in Appendix 11.

**Table 4.19: Mean posterior parameter estimates and 95% Bayesian Credibility Intervals (BCI) for the final caprine model using the relaxed prior set\* and assuming conditional dependence**, *Se is sensitivity, Sp is specificity, RBT is Rose Bengal plate test and cELISA is competitive enzyme-linked immunosorbent assay, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive*

| Parameter | Mean | 2.5% BCI | 97.5% BCI |
|---|---|---|---|
| Se: RBT | 0.820 | 0.612 | 0.984 |
| Sp: RBT | 0.991 | 0.981 | 1 |
| Se: cELISA | 0.595 | 0.432 | 0.736 |
| Sp: cELISA | 0.986 | 0.976 | 0.997 |
| Se: series | 0.489 | 0.294 | 0.648 |
| Sp: series | 1 | 1 | 1 |
| Se: parallel | 0.926 | 0.807 | 0.994 |
| Sp: parallel | 0.977 | 0.959 | 0.995 |
| Prevalence: exclusive pastoralist subpopulation | 0.053 | 0.035 | 0.075 |
| Prevalence: non-exclusive pastoralist subpopulation | 0.009 | 0.000 | 0.023 |
| covDn | 0.007 | 0.000 | 0.015 |
| covDp | -0.009 | -0.075 | 0.077 |

\*relaxed prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(1,1)

**Table 4.20: Positive and negative predictive values for RBT, cELISA, in-series and in-parallel diagnostic approaches in an exclusive pastoralist subpopulation for the final ovine model**, *RBT is Rose Bengal plate test and cELISA is competitive enzyme-linked immunosorbent assay, in-series is testing all RBT positive samples by cELISA, in-parallel is testing all samples by RBT and cELISA*

| Diagnostic approach | Positive Predictive Value | Negative Predictive Value |
|---|---|---|
| RBT | 0.836 | 0.990 |
| cELISA | 0.704 | 0.978 |
| In-series | 1 | 0.972 |
| In-parallel | 0.693 | 0.996 |

## 4.3.8 Final model estimates for all livestock models

Sensitivity, specificity and prevalence estimates for each diagnostic test for the final bovine model with conditional independence and relaxed prior set and ovine and caprine models with conditional dependence and relaxed prior sets are shown in Figure 4.11. The posterior mean estimates and 95% BCI for in-series and in-parallel diagnostic testing approaches for the final bovine, ovine and caprine models are shown in Figure 4.12.
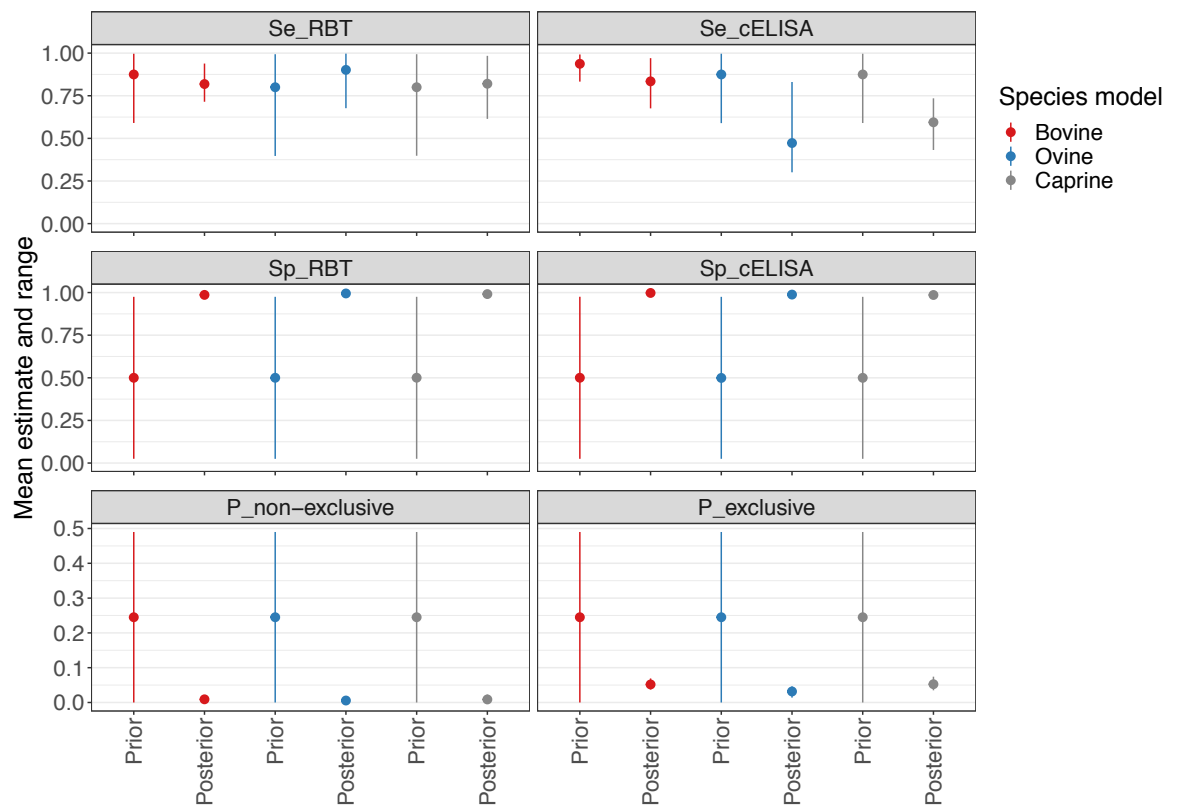


**Figure 4.11: Sensitivity, specificity and prevalence posterior mean estimates and ranges for the final bovine model with relaxed priors and conditional independence (without covariance) and the final ovine and final caprine models with relaxed prior sets and conditional dependence (with covariance)**, *Se is sensitivity, Sp is specificity, RBT is Rose Bengal plate test, cELISA is competitive enzyme-linked immunosorbent assay, P is prevalence, non-exclusive is the non-exclusive pastoralist subpopulation and exclusive is the exclusive pastoralist subpopulation*
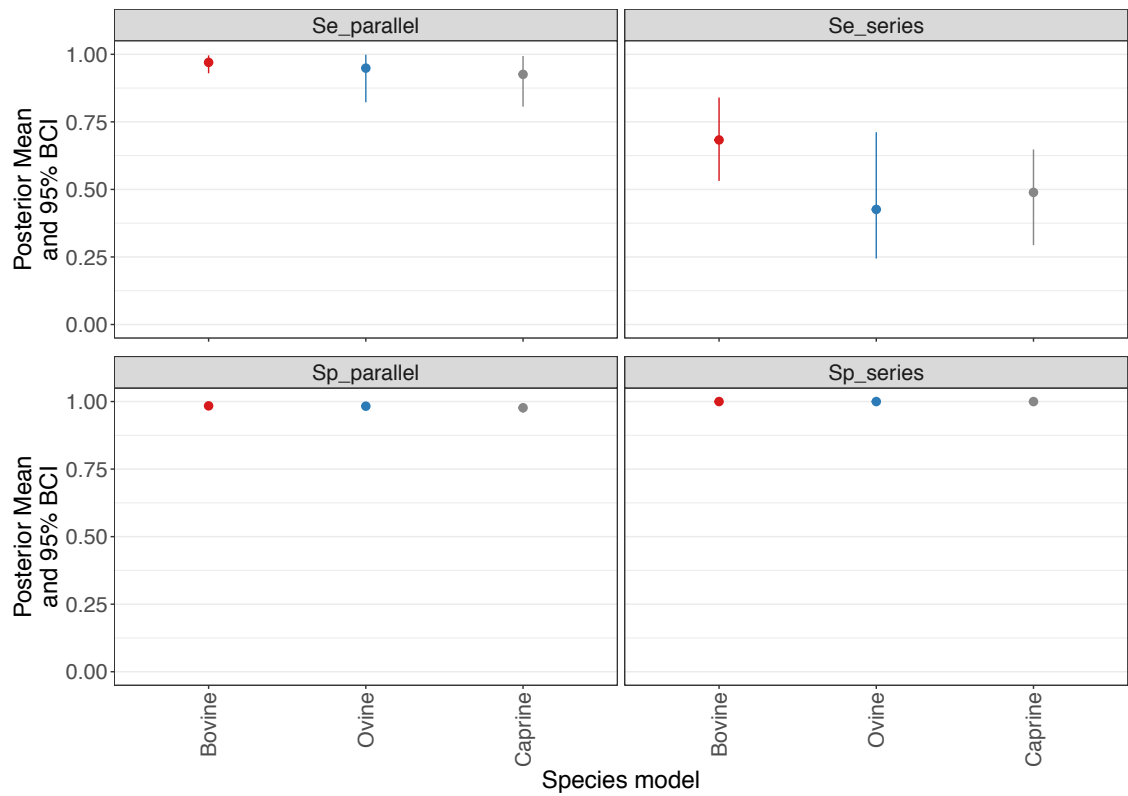
**Figure 4.12: In-series and in-parallel sensitivity and specificity posterior mean estimates and 95% Bayesian credibility intervals (BCI) for the final bovine model with relaxed priors and conditional independence (without covariance) and the final ovine and final caprine models with relaxed prior sets and conditional dependence (with covariance)**, *Se_parallel is in-parallel sensitivity, Se_series is in-series sensitivity, Sp_parallel is in-parallel specificity, Sp_series is in-series specificity*

## 4.4 Discussion

There is no standardised diagnostic testing approach for animal brucellosis in Tanzania (Government of Tanzania, 2018a). Evaluations of the performance of diagnostic tests are crucial in gaining a true understanding of the epidemiology of brucellosis and to inform effective disease surveillance and control strategies. Here, the performance of two serological tests for brucellosis: RBT and cELISA, were evaluated for naturally exposed cattle, sheep and goats in northern Tanzania using Bayesian latent class analyses. Sensitivity was variable across livestock models; RBT sensitivity was comparable to cELISA in the bovine model and greater than cELISA in ovine and caprine models. RBT and cELISA specificity was comparable across livestock models. Conducting RBT and cELISA testing in parallel optimised diagnostic test performance in all livestock models.

The final bovine model included relaxed priors guided by the available literature on RBT and cELISA test performances for cattle in Africa. The same was true for ovine and caprine models, except priors were guided by literature from studies outside Africa. The RBT and cELISA sensitivity priors were informative for all livestock species final models, as posterior estimates had wide credibility intervals without informative priors. This was likely because of the low number of sample positives for each livestock species. RBT and cELISA specificity had uniformly distributed priors specified in all livestock species final models, as the posterior estimates remained consistently high without informative priors. This was expected to be in part due to the large sample size available for each species.

For the bovine model, the inclusion of covariance resulted in a marginally different DIC to the model without covariance. This indicated a minimal correlation between RBT and cELISA tests for cattle, so the simpler conditional independence model was preferred. For both the ovine and caprine models, DIC values indicated correlation between RBT and cELISA. Therefore, the ovine and caprine models assuming conditional dependence were preferred over the simpler models. The issue of assuming conditional independence or dependence is not straightforward, as diagnostic tests that identify similar responses to disease are likely all dependent to some extent (Branscum et al., 2005). In the case of RBT and cELISA it seems likely that there is dependence as both are

serological tests detecting antibodies to *Brucella* smooth lipopolysaccharides. However, serological tests such as these differ in the isotypes detected and isotype-specific properties also differ during the course of infection, altering detectability (Ducrotoy et al., 2018). Therefore, due to varying isotype ratios and properties, dependence between these tests may not be constant throughout *Brucella* spp. infection (Ducrotoy et al., 2018).

For the final bovine model, the mean posterior RBT sensitivity estimate was in accordance with other studies in Africa (Chisi et al., 2017; Etman et al., 2014; Getachew et al., 2016; Hosein et al., 2017; Matope et al., 2011; Muma et al., 2007; Sanogo et al., 2013). However, the cELISA estimate for this study was lower than estimates reported for previous studies (Bronsvoort et al., 2009; Chisi et al., 2017; Etman et al., 2014; Matope et al., 2011; Muma et al., 2007). As mentioned earlier, posterior estimates for sensitivity may have been low due to a small number of positive animals in the dataset, which is a consequence of the generally low-level prevalence nature of *Brucella* spp. in naturally infected livestock. The final bovine model mean posterior estimates for RBT and cELISA specificity place this study towards the high end of the reported specificity range for cattle in Africa (Bronsvoort et al., 2009; Chisi et al., 2017; Etman et al., 2014; Getachew et al., 2016; Hosein et al., 2017; Matope et al., 2011; Muma et al., 2007; Sanogo et al., 2013).

For the final ovine and caprine models the RBT sensitivity mean posterior estimates were positioned towards the top of the estimate range identified for other small ruminant test performance studies outside of Africa (García-Bocanegra et al., 2014; Gupta et al., 2014; Marín et al., 1999; Minas et al., 2008, 2005; Nielsen et al., 2004; Patel et al., 2017; Rahman et al., 2013; Ramírez-Pfeiffer et al., 2008).The cELISA sensitivity posterior estimates for the ovine and caprine models in this study were lower than the identified literature estimate range (Burriel et al., 2004; García-Bocanegra et al., 2014; Marín et al., 1999; Minas et al., 2008, 2005; Nielsen et al., 2005, 2004), as was found for cattle also. For the ovine and caprine models in this study, both RBT and cELISA specificity mean posterior estimates are positioned towards the top of the identified estimate range from previous studies (Burriel et al., 2004; García-Bocanegra et al., 2014; Gupta et al., 2014; Marín et al., 1999; Minas et al.,

2008, 2005; Nielsen et al., 2005, 2004; Patel et al., 2017; Rahman et al., 2013; Ramírez-Pfeiffer et al., 2008). Interestingly, it is generally considered that the cELISA is a more specific test than the RBT (Makita et al., 2011a; OIE, 2018). However, in this study RBT outperformed the cELISA in both the ovine and caprine models.

Across the livestock models in this study, sensitivity was the most variable test performance measure, particularly for cELISA (0.472 – 0.835). These results suggest that running the cELISA test alone would create a high proportion of false negatives, especially in sheep samples. RBT sensitivity was less variable and greater across livestock models (0.819 – 0.902) as compared to cELISA. In contrast to cELISA, RBT sensitivity was marginally higher in sheep samples than cattle and goats. Specificity for both RBT and cELISA was consistently high across livestock models. These results are important in informing an evidence-based diagnostic testing approach for livestock brucellosis in Tanzania. As an initial screening or single test in routine surveillance, cELISA is not recommended in this setting. The results of this study indicate that RBT performs more consistently and with higher sensitivity as a single testing approach across cattle, sheep and goat samples as compared to cELISA.

In this study, the bovine model estimated brucellosis seroprevalence at 5.2% (95% BCI: 3.7% - 6.9%) and 1.0% (95% BCI: 0.4% - 1.6%) for the exclusive pastoralist and non-exclusive pastoralist subpopulations, respectively. Ovine model seroprevalence posterior estimates were 3.2% (95% BCI: 1.4% - 4.6%) and 1.0% (0.0% - 1.5%) for exclusive and non-exclusive pastoralist subpopulations, respectively. Caprine model seroprevalence posterior estimates were 5.3% (95% BCI: 3.5% - 7.5%) and 1.0% (95% BCI: 0.0% - 2.3%) for exclusive and non-exclusive pastoralist subpopulations. These prevalence estimates are within the reported estimate species ranges for other studies in Tanzania (Assenga et al., 2015; Chitupila et al., 2015; John et al., 2010; Lyimo, 2013; Mathew et al., 2015; Mellau et al., 2009; Sagamiko et al., 2018; Shirima et al., 2010; Swai and Schoonman, 2010). The ovine model had the lowest prevalence estimates of three livestock species. Caprine and bovine models had similar prevalence estimates. It has been reported in SSA that the prevalence of ovine or caprine brucellosis is generally lower than bovine brucellosis (Mcdermott and Arimi,

2002). Posterior prevalence estimates in this study do not indicate a substantial difference between these livestock species. Across all livestock species models, brucellosis prevalence was estimated to be highest in the exclusive pastoralist subpopulation. This is in agreement with previous studies where prevalence has been estimated to be higher in exclusive pastoral areas as compared to non-exclusive pastoral areas, such as agro-pastoralist or smallholder populations (Mcdermott and Arimi, 2002; Shirima et al., 2010; Swai and Schoonman, 2010). A higher prevalence in exclusive pastoral settings, as compared to other settings, is reported to be attributable to larger herds or flocks, mixing of livestock species and higher contact rates between animals (Mcdermott and Arimi, 2002; Racloz et al., 2013). Additionally, exclusive pastoralist communities are more likely to be involved in transhumance. These national and cross-border livestock movements, as well as trade, are important factors facilitating livestock disease transmission, including brucellosis (Dean et al., 2013b, 2013a).Therefore, prioritising brucellosis prevention and control activities in exclusive pastoralist communities in Tanzania may achieve the most effective reduction in brucellosis prevalence.

There is no single recommended serological test for the diagnosis of brucellosis and therefore the use of a screening test in combination with a confirmatory or complementary test is advisable (OIE, 2018). In this study, in-series and in-parallel, as well as single diagnostic testing approaches for RBT and cELISA were assessed. Sensitivity and specificity estimates and PPVs and NPVs under each of the diagnostic approaches were compared. An increased probability of an animal that is truly brucellosis negative testing negative (high NPV) is important in classifying that an individual or herd/flock is truly disease-free. During routine national surveillance that would be used to guide prioritisation of national brucellosis prevention and control interventions in high-risk areas, a high NPV at the individual or herd/flock level is arguably more important than an improved probability of truly disease positive animals testing positive. This is because the ability to classify disease-free status would allow more precise prioritisation of areas that require immediate brucellosis prevention and control intervention resources. In contrast, a high PPV may be most important when surveillance is conducted for the purpose of providing information on individual animal disease status, for example in giving feedback to livestock-keepers. A high PPV may be

more important in moving towards brucellosis eradication and the ability to successfully classify individual animals as truly disease positive. Eradication cannot be considered until progress has first been made in the prevention and control of brucellosis. Here, the diagnostic testing approach with a high combined sensitivity and specificity, as well as high NPV was selected as the most effective diagnostic approach for use in routine brucellosis surveillance and informing national prevention and control interventions. In-series specificity posterior mean estimates were high for all livestock species models. However, as expected with this diagnostic approach sensitivity was reduced, and substantially so, as compared to the in-parallel approach. Running the two tests in-parallel gave an improved mean posterior estimate for sensitivity and the in-parallel specificity was only marginally reduced for each livestock species model as compared to in-series testing. In-parallel diagnostic test performance was comparable across all livestock species, with the highest sensitivity in the bovine model 0.970 (95% BCI: 0.930 – 0.996) and the lowest in the caprine model 0.926 (95% BCI: 0.807 – 0.994). In-parallel specificity was consistently high with all livestock models indicating 0.977 (95% BCI: 0.959 – 0.995) or greater. Although the PPV was lowest for an in-parallel testing approach, NPV was higher for in-parallel as compared to in-series testing for all livestock models. A testing protocol applying RBT and cELISA in-parallel for bovine, ovine and caprine brucellosis in Tanzania would be an ideal approach for use in routine national surveillance in order to classify individual animals or herds/flocks as truly disease-free, and guide prioritisation of prevention and control interventions.

There is no nationally adopted diagnostic testing strategy for brucellosis in Tanzania (Government of Tanzania, 2018a), suggested diagnostic tests include initial screening by RBT and confirmation by ELISA, or PCR or culture (Government of Tanzania, 2018b). The RBT is a recognised screening test for livestock brucellosis, and the iELISA is the OIE recommended ELISA for confirmatory testing (OIE, 2018). Here, the cELISA was investigated as a confirmatory test, as it was considered a more practical test to implement in Tanzania. The cELISA can be used for multiple host species, whereas variants of the iELISA are host species-specific, as well as immunoglobulin-specific. The iELISA is also a more expensive test per sample (APHA, 2020). However, it has been recognised that the application of cELISA testing in LMICs may also be

challenging, as it requires equipment that may not be routinely accessible (Matope et al., 2011). The cELISA is available in a limited number of laboratories in Tanzania but is not routinely used. Currently, animal samples would need to be received by centralised laboratories for testing, which would delay test turnaround times. Nevertheless, this study indicates that the cELISA can be an invaluable confirmatory test to the RBT.

In Tanzania, the national guidelines for surveillance of the prioritised zoonotic diseases indicates the need for animal brucellosis active surveillance in the form of mass screening (Government of Tanzania, 2018b). If mass animal brucellosis screening in Tanzania were to be conducted, applying the results of this study would be problematic as parallel testing every individual animal by both diagnostic tests would require significant resources, including increasing regional-level laboratory capacity for cELISA diagnostics. The diagnosis of brucellosis at the herd- or flock-level is expected to be more useful in terms of surveillance for disease prevention and control, as opposed to diagnosis at the individual animal level (Corbel, 2006; OIE, 2018). Herd/flock testing would involve testing a proportion of animals in every herd/flock; this would reduce resource requirements yet still enable national-scale data collection. In order to reduce resource requirements further, a pragmatic approach could be to run RBT and cELISA in-parallel on a single pooled sample from a herd/flock. This pooled sample would be taken from a proportion of individuals and would require a single RBT and cELISA test per herd/flock. Pooled sample testing to determine the herd/flock disease status can be more cost-effective than individual animal testing when disease prevalence is expected to be less than 10% (Cowling et al., 1999). Additionally, focusing resources and prioritising surveillance in exclusive pastoralist populations, where seroprevalence has been shown to be higher than other livestock-keeping populations, may help to understand the true burden of brucellosis and the effects of prevention and control activities. RBT testing could be conducted by trained local government veterinary representatives in the field with standardised reagents. Diagnostic testing capacity could be strengthened to provide trained personnel and standardised cELISA equipment at the regional government veterinary laboratories. This approach would allow for animal brucellosis screening by RBT in the field and complementary in-parallel testing by cELISA in selected

laboratories. Which has been indicated in this study as the diagnostic testing approach with the greatest test performance as compared to using either test alone, or in-series diagnostic test approaches.

A limitation of the model implemented here was that it included two serological tests in two subpopulations, which was not ideal for the inclusion of a conditional dependence parameterisation (Toft et al., 2005). Further study may benefit from collecting different samples from the same animal such as sera and milk and to test these by both serological and molecular approaches, such as PCR. It may be possible to then implement a latent class model with RBT and PCR or cELISA and PCR, where the assumption of conditional independence may be better satisfied. Additionally, model assumption (b) that diagnostic test performance is the same across subpopulations could be investigated further. It is possible that as brucellosis prevalence is variable in different subpopulations, that pathogens causing serological cross-reactions may also be variable. This could therefore affect test performance in the different livestock subpopulations sampled.

## 4.5 Conclusion

This is the first study to evaluate the test performance of RBT and cELISA for detecting bovine brucellosis in Tanzania and is likely the first for ovine and caprine brucellosis in Africa by Bayesian latent class analysis. Sensitivity of test performance varied for RBT (0.819 – 0.902), and even more so for the cELISA (0.472 - 0.835), in all livestock models. RBT sensitivity was comparable to cELISA in the bovine model and greater than cELISA sensitivity in the ovine and caprine models. RBT and cELISA specificity was consistently high for all livestock models (0.986 - 0.995) (0.986 - 0.998), respectively. The prevalence of brucellosis was higher for the exclusive pastoralist subpopulation than the non-exclusive pastoralist subpopulation for bovine, ovine and caprine models, reinforcing that the burden of brucellosis is greater in exclusive pastoralist communities. In the detection of bovine, ovine and caprine brucellosis, conducting RBT and cELISA parallel testing optimised diagnostic test performance, as compared to using either test alone or a series approach. These test performance and prevalence findings can assist in the development of a national disease surveillance strategy

that can generate robust true burden estimates for brucellosis in Tanzania and wider SSA. The implementation of an effective and standardised approach to the detection of animal brucellosis is a crucial step towards the control and prevention of brucellosis in both animals and humans.

# Chapter 5 Discussion

Brucellosis is a "difficult disease" for many reasons, including the non-distinguishing and inconsistent clinical symptoms in both humans and animals, the variable performance of brucellosis diagnostic tools, and the presence of multiple pathogen species infecting multiple animal host species (Ducrotoy et al., 2017). More robust epidemiological data are required in order to better understand the true burden of brucellosis and to inform the development of effective disease prevention and control interventions (Dean et al., 2012b). This is especially true for sub-Saharan Africa (SSA), where the global burden of brucellosis is estimated to be greatest (Racloz et al., 2013). Pastoralist communities in particular are suspected to carry a high brucellosis burden, with people suffering from regular *Brucella* spp. exposures (Mcdermott and Arimi, 2002). The overarching aim of this thesis was to improve our understanding of the epidemiology of brucellosis in Tanzania, with a focus on human brucellosis in pastoralist populations and diagnostic test performance in animal hosts.

## 5.1 The current brucellosis prevention and control strategy in Tanzania

In Tanzania, brucellosis has been recently identified by multisectoral collaboration as one of six national priority zoonotic diseases (Government of Tanzania, 2018b). In 2018, a five-year national strategy for brucellosis prevention and control in humans and animals was also released, which highlights the strengths and weakness of the situation in Tanzania with regards to brucellosis prevention and control (Government of Tanzania, 2018a). The challenges to brucellosis prevention and control in Tanzania are multifaceted, involving public health and animal health, as well as sociological and even environmental considerations. In the national strategy for brucellosis areas for improvement have been identified, such as the need for: detailed epidemiological data and a national research agenda; development of effective surveillance and diagnostics; consideration of community socio-cultural values; development of policy guidelines for control; and multi-sectoral communication and collaboration (Government of Tanzania, 2018a). The formulation of this national strategy is a fundamental step towards implementing a standardised and effective brucellosis prevention and control approach in Tanzania.

## 5.2 The importance and application of identified risk factors for human brucellosis

Understanding the risk factors for acute human infection in pastoralist communities would assist in addressing the knowledge gap regarding the need for robust data on the transmission of brucellosis, particularly in areas suspected of high disease burden. These data can be used in the prioritisation of a human risk demographic for the implementation of a brucellosis prevention and control intervention. Prevention and control interventions that focus on setting-specific human risk activities can rapidly reduce the incidence of human brucellosis. An example of this is the reduction in incidence of human brucellosis in Greece, following milk pasteurisation and increased hygiene practices in the production of feta cheese (Minas et al., 2004). In Chapter 2, two risk factors were identified for acute brucellosis in febrile patients attending a rural hospital in Tanzania. This is the first study to identify risk factors for acute brucellosis in a pastoralist community of Tanzania, and one of few in East Africa. Being a young member of the community and involved in herding cattle, sheep or goats was associated with an increased risk of acute brucellosis. These findings provide vital information on who in the human population of the Ngorongoro Conservation Area (NCA) is at a high-risk of acute infection. If incorporated into a disease prevention and control strategy, these findings have the potential to substantially reduce the burden of acute brucellosis in the NCA. Specifically, by identifying and targeting young herders for communication about brucellosis transmission, this can address the need for better awareness of brucellosis risk in livestock keepers, as identified by the national brucellosis strategy (Government of Tanzania, 2018a). Rather than continuing with risk factor analyses of various strata in this community, the next step to further these findings would be to identify the risk behaviours being conducted by young members whilst herding. Following this, sensitisation of this demographic to the identified transmission risks for brucellosis would allow people to make informed decisions about brucellosis risk behaviour and could result in a substantial reduction in the burden of brucellosis. However, it must be recognised that an approach such as this to disease prevention must consider community-specific cultural traditions and perceptions (Marcotty et al., 2009). A successful strategy in these settings, particularly one based on behavioural changes, must be co-

developed in collaboration with the community at risk including key influential community representatives.

## 5.3 The importance and feasibility of active surveillance techniques

In resource-limited settings, brucellosis prevention and control interventions have to be targeted in their approach and must identify priority areas for intervention. This prioritisation cannot be informed by passive surveillance data using healthcare facility records only. This is due to a number of reasons, including the lack of a standardised, consistent brucellosis reporting system for healthcare facilities in Tanzania (Government of Tanzania, 2018a). Additionally, in SSA only a proportion of the population in ill-health ever reach a healthcare facility (Panzner et al., 2016), meaning hospital records are not representative of the true brucellosis burden. There are various barriers to accessing healthcare. For example, approximately 25% of the total population live more than 2 hours travel from the nearest hospital providing emergency care in Tanzania (Ouma et al., 2018). Although emergency care is not directly comparable to healthcare facility requirements for brucellosis treatment, the study gives an approximation of a barrier to care faced across Tanzania. This barrier is likely only enhanced in geographically remote pastoralist communities. The use of cross-sectional community-based active surveillance also has disadvantages, including the high cost of collecting limited information on brucellosis burden due to the suspected non-uniform distribution and grouped nature of acute brucellosis infections. The national strategy for Tanzania identifies a lack of representative surveillance data that can be used to inform prevention and control of brucellosis (Government of Tanzania, 2018a). Chapter 3 attempted to address the surveillance knowledge gap in order to assist in understanding the true burden of brucellosis in Tanzania. An adapted active surveillance technique was investigated to assess its ability to detect additional acute brucellosis cases in household members of febrile individuals reaching hospital. In the households of febrile patients with acute brucellosis, additional acute brucellosis cases were detected in household members. Yet, the study did not show evidence of significant grouping between febrile patients with acute brucellosis and household members exposed to *Brucella* spp. With a larger

sample size and longer timeframe, this study may have been sufficiently powered to show a significant association between febrile hospital patients with acute brucellosis and their household members. However, given the challenges of gathering these data through a well-resourced research study, this form of active surveillance does not appear to be a feasible, nor sustainable approach to additional case finding in the community and estimation of the true burden of brucellosis. Not only did this active case finding approach require a diverse team of specialists to implement, but sample size was severely limited by the high rate of study participation decline by the febrile patient population.

There are alternative community-based approaches that may be more effective in obtaining accurate data on the burden of brucellosis in pastoralist communities and could optimise resource use and study participation rates. A linked hospital-based surveillance study collected data on the prevalence of acute brucellosis in febrile hospital patients (Bodenham et al., 2020). An alternative approach is to combine these prevalence data with a healthcare utilisation survey. A healthcare utilisation survey takes a stratified sample of households in the catchment area of a healthcare facility and administers a questionnaire at each household about household member healthcare seeking behaviour. Using the hospital and community-acquired data, multipliers can then be used to estimate the incidence of brucellosis. A community-based active surveillance approach such as this requires a less specialised field team. Study participation is also likely to be higher as only one member of the household need participate and no physically invasive sampling is required. This approach to understanding the true burden of brucellosis has already been successfully implemented in Moshi, Tanzania (Carugati et al., 2018) and is underway in the NCA. However, robust data on the prevalence of acute brucellosis in a febrile population attending a healthcare facility is a necessary prerequisite for this approach and is clearly not readily available. Another possible active surveillance approach for human brucellosis could be to adapt a community-based system that is already in place. A study using a participatory approach in rural Nepal trained pre-existing community health workers to collect syndromic data using a smart phone application during their routine community visits (Meyers et al., 2016). In Tanzania, community health workers and also community outreach clinics are part of the broader healthcare system. There

are also at least two examples of beta smart phone applications that have been developed for the collection of public and animal health data, and have been implemented in Tanzania: AfyaData, an open source tool developed by the Southern African Centre for Infectious Disease Surveillance (SACIDS); and the Events Mobile Application (EMA-*i*), an animal health reporting tool developed by the FAO. In Tanzania, local community health workers as well as other healthcare facility community outreach teams could be trained to collect data in the community on febrile illness and healthcare seeking behaviour using an adaptation of an existing mobile data collection platform. This could prove to be a cost- and time-effective approach to active surveillance for brucellosis, when used alongside hospital-based passive surveillance in Tanzania. A combined hospital and community-based approach such as this could help address the need for representative brucellosis surveillance data in Tanzania (Government of Tanzania, 2018a). However, in order to implement such a surveillance approach, it is first necessary to develop a standardised brucellosis case definition and diagnostic testing procedure, so that brucellosis can be correctly diagnosed. An evaluation of the human brucellosis diagnostics available in healthcare facilities in Tanzania is currently underway.

## 5.4 The importance of evaluating diagnostic test performance and next steps

Evaluation of the performance of available diagnostic tools for brucellosis is crucial in understanding the true burden of disease in humans and animals, and also reliably monitoring changes in disease burden following implementation of prevention and control interventions. The Tanzanian national strategy for brucellosis prevention and control identifies a need for standardised and validated tests for the diagnosis of brucellosis (Government of Tanzania, 2018a). A linked study to the research presented in this thesis has isolated and characterised both *B. melitensis* and *B. abortus* in the febrile hospital population of the NCA (Bodenham et al., 2020). It is therefore particularly important to evaluate the performance of diagnostic testing protocols for cattle, sheep and goats in Tanzania, as each species likely has a role as a maintenance host for brucellosis. Chapter 4 attempted to address the knowledge gap regarding the need for a standardised and validated testing procedure for the

diagnosis of animal brucellosis in Tanzania, by evaluating the performance of the RBT and the cELISA as: individual tests; run in series; or run in parallel of one another. Diagnostic test evaluation was performed for each livestock species individually. This study was the first RBT and cELISA test performance evaluation for livestock brucellosis in Tanzania and is believed to be the first for small ruminants in SSA. Aside from generating sensitivity and specificity estimates for the RBT and cELISA individually, test performances were estimated to be optimal when run in-parallel for each livestock species. Applying the results of this study to mass animal surveillance in Tanzania is challenging as testing every individual animal by both diagnostic tests would require significantly more resources. A more practical and cost-effective approach may be to implement herd- or flock-level testing using a single pooled sample from each herd/flock. Examples of pooled testing of animal samples are infrequent (Cowling et al., 1999), some examples include faecal samples in detection of *Mycobacterium paratuberculosis* and *Salmonella* spp. (Jordan, 2005; Wells et al., 2003). In order to consider the application of a pooled sample approach for livestock brucellosis, it would be necessary to conduct a sensitivity analysis in order to estimate the optimal pool size for detection of *Brucella* spp. antibodies, such as that conducted for pooled sera in the detection of *Schistosoma japonicum* (Jia et al., 2009). It would be equally important to generate cost estimates for a pooled approach under varying brucellosis prevalence estimates, such as that performed for pooled blood in detecting bovine viral diarrhoea virus (Muñoz-Zanzi et al., 2000). Additionally, exploring the performance of varying combinations of in series and in parallel diagnostic testing during surveillance may be valuable in reducing resource requirements. Analyses could be performed in order to understand test performance using primarily series testing with a set proportion of parallel testing sites.

## 5.5 Brucellosis knowledge gaps

Overall, the three core thesis chapters have attempted to address the following brucellosis knowledge gaps in Tanzania: 1 – the need for more detailed epidemiological data, particularly in areas suspected to be at high risk of brucellosis; 2 – the need for surveillance approaches that can assist in providing a true representation of brucellosis burden; and 3- the need for a validated and

standardised brucellosis diagnostic test approach. With regards to these knowledge gaps, findings have been presented on: 1- risk factors for acute human infection in a high-risk community; 2 - an adapted active surveillance technique for the detection of acute human infections and exposure to *Brucella* spp.; and 3 - diagnostic test performance and estimation of the prevalence of brucellosis in different livestock species. These findings together contribute towards the need for robust epidemiological data in order to improve our understanding of the true burden of brucellosis in Tanzania and wider SSA.

Following the collection of data for Chapter 2 and 3, there are features of study design and implementation that should be considered in future research. Risk factor data collection was a paper-format questionnaire and lasted approximately 40 minutes per participant. Forty minutes was too long, with some participants failing to complete all sections. Streamlining the number of questions would be an advantage. Also, switching to a digital format might increase the speed of data collection and would reduce administrator errors, such as skipping questions. An overlooked limitation of household member sampling was that more than half of households would decline participation in the study. This was ultimately a limiting factor to the power of the study. Febrile patients were informed at hospital enrolment that they may be contacted for a household visit. Possibly, the provision of an information leaflet to take away and show to other family members may have increased household participation. A proportion of household participation declines were due to absence of the head of household. The household sampling period was largely during the dry season. In this community, male household members (including the head of household) may travel for improved livestock grazing or work purposes during this season. Therefore, socio-cultural aspects of this community had a large impact on study implementation.

## 5.6 Future research

Some further knowledge gaps that need to be addressed in future research in order to implement an effective brucellosis prevention and control strategy in Tanzania include: identification of a rapid, standardised and validated diagnostic testing approach for human and animal brucellosis, and subsequently

identification of a national human and animal case definition; investigation into strengthening passive surveillance reporting channels so that more representative data are available; further research into appropriate community-based active surveillance approaches; and identification of a cost-effective, sustainable approach to vaccination of cattle, sheep and goats. A prevention and control measure that can be considered for rapid implementation is communication about brucellosis transmission directed at high-risk individuals in livestock-keeping communities. As well as, the collaborative development and implementation of setting-specific brucellosis hygiene measures in these communities.

## 5.7 Suggestions for the implementation of brucellosis prevention and control in Tanzania and SSA

### 5.7.1 An ideal brucellosis prevention and control strategy

There are a range of different activities that have been implemented in various countries around the world in order to prevent and control brucellosis. In Tanzania and wider SSA, it essential that the strategy implemented is appropriate for the setting in which it will be applied. Prevention and control activities routinely used in high-income settings, such as: test and slaughter; restricting livestock movements; and financial incentives for disease-free herd status, would likely prove difficult to implement, monitor and sustain in the SSA. Intervention using livestock vaccination could be an effective approach in the reduction in prevalence of brucellosis in livestock and people, and has been suggested as such for SSA (WHO et al., 2006). In an ideal scenario, such an intervention in Tanzania would include routine mass vaccination of cattle, sheep and goats, at a very low cost per animal to the livestock keeper. Based on current guidance regarding the most effective brucellosis vaccines, vaccination would involve the use of S19 vaccine for cattle and Rev-1 vaccine for sheep and goats (Corbel, 2006). The conjunctival vaccination of calves, lambs and kids only is suggested and would need to be conducted at less than 4 months old, with revaccination within 6-12 months of initial vaccination. This vaccination approach is advocated as it would avoid the abortifacient effect induced by

these vaccines in adult animals and reduces interference with serological testing (Godfroid et al., 2011). Vaccination would require individual animal identification for trace-back, potentially using ear tags. Additionally, appropriate training and personal protective equipment for veterinary service providers would be crucial in ensuring that the occupational risk of vaccine administration is as minimal as possible. This vaccination strategy could also be combined with community knowledge exchange concerning risk factors for animal and human brucellosis and the hygiene measures that might be feasibly implemented to prevent transmission. This would include food safety measures, such as milk boiling, that have been key in the prevention of human brucellosis in countries such as Greece, and are thought particularly imperative for the prevention and control of *B. melitensis* in LMICs (Godfroid et al., 2013a). Implementation of hygiene measures with regard to livestock-wildlife interface areas, such as the avoidance of shared livestock and wildlife grazing areas (Van Campen and Rhyan, 2010), would likely also be important. This brucellosis prevention and control strategy would assist in reducing brucellosis transmission and the burden of infection in animals and humans. However, it would require consideration of the most effective method of communicating and exchanging information for different demographic groups. This would be important in having the highest chance of effecting behavioural change in livestock keeper compliance with a vaccination strategy, and also in the avoidance of identified risk factors, including successful implementation of food safety measures.

### 5.7.2 Considerations for a successful brucellosis prevention and control strategy

Through the progression of this thesis research, some important points concerning brucellosis prevention and control in Tanzania have become apparent. As an NZD with very low human mortality rates, it is important to recognise that a brucellosis prevention and control strategy will need to be justifiable within the greater landscape of human disease burden in SSA. As has been suggested for the control of NZDs and NTDs in LMICs, one method of achieving the successful adoption of a brucellosis prevention and control strategy may be to involve the integration of several diseases (Molyneux et al., 2005; WHO et al., 2006). This multi-disease control approach could be adapted

via the inclusion of other NZDs that occupy a similar ecological setting and cause similar syndromic presentation in humans, such as Q fever (*Coxiella burnetii*) and leptospirosis (*Leptospira* spp.). In Tanzania, the government has produced documentation identifying six priority zoonotic diseases including: brucellosis; rabies; avian influenza; anthrax (*Bacillus anthracis*); human african trypanosomiasis (*Trypanosoma brucei* spp.); and Rift Valley fever and other viral haemorrhagic fevers (Government of Tanzania, 2018b). For prioritised zoonoses involving similar host species and found in a similar geographical setting to brucellosis, such as anthrax, certain aspects of a disease prevention and control strategy could be combined into a multi-disease approach. These aspects might include: communication about transmission risk in livestock-keeping communities; or the implementation of a multi-disease mass vaccination campaign, if an effective vaccination strategy can be agreed for brucellosis in Tanzania. Additionally, communication about brucellosis transmission with regards to food safety measures such as boiling milk and cooking meat appropriately would impact on the burden of multiple zoonoses, for example: tuberculosis (*Mycobacterium bovis*); Q fever; *Salmonella* spp.; *Escherichia coli*; and *Campylobacter* spp.

Secondly, as suggested in previous reports focusing on the control of NZDs, a strategy should be oriented around a One Health approach, which depends upon involvement and effective communication between public health, animal health and sociological sectors (Godfroid et al., 2013a; Government of Tanzania, 2018a; WHO et al., 2006). In pastoral settings, environmental factors may also need to be taken into account, with control considering impacts on issues such as: protection of ecosystem services; land reform; and integrated social and economic development (Racloz et al., 2013). The adoption of technology such as smart phone applications in the surveillance of brucellosis and other NZDs can only help to facilitate communication between multi-sectoral stakeholders. Data are uploaded to a central server, which would facilitate formal multi-sectoral reporting to stakeholders. Such surveillance data could also assist in reporting between bordering countries. This is important in brucellosis prevention and control, as bordering countries that share transhumance routes most likely also share brucellosis burden (Dean et al., 2013a).

Finally, when developing disease prevention and control strategies it is important that any strategy is informed by an evidence-base of robust data. Without this, any strategy will likely be ineffective or even harmful (Godfroid et al., 2013a). The study findings presented in this thesis contribute towards this evidence-base regarding the epidemiology of brucellosis in Tanzania and SSA. It is however necessary to ensure that this evidence-base is effectively translated, so that the core messages are accessible and clear to stakeholders without a scientific background. Therefore, an important next step is to develop a concise policy brief that summarises the key findings from the research presented here, as well as combining research outcomes from other available studies, such as data on human brucellosis incidence rates and the *Brucella* species causing human infections in Tanzania. The policy brief could suggest appropriate next steps in planning for the implementation of prevention and control activities, such as trailing livestock vaccination and investigating steps towards the sustainable implementation of a routine vaccination campaign through government collaboration with appropriate international organisations such as Gavi, The Vaccine Alliance. Additionally, advocating for investigation into a number of different costing analyses would provide important information regarding the feasible application of brucellosis prevention and control activities. These costing analyses could include: evaluation of the cost-effectiveness of routine livestock brucellosis vaccination on human and animal brucellosis control, as has been conducted in Tanzania for other infectious diseases such as rabies, rotavirus and malaria (Fitzpatrick et al., 2014; Hutton and Tediosi, 2006; Ruhago et al., 2015); cost-benefit analysis of a national standardised livestock brucellosis diagnostic testing protocol for brucellosis surveillance and control; and a cost-benefit analysis evaluating a multiple zoonoses approach to a prevention and control strategy, particularly in the case of the six prioritised zoonoses for Tanzania. This communication with policy-makers could assist in obtaining investment in essential research and generating momentum towards adoption of an evidence-based brucellosis prevention and control strategy for Tanzania. These multiple steps taken towards the successful prevention and control of brucellosis in Tanzania could be used to guide prevention and control activities in similar settings across SSA.

# Appendices

**Appendix 1: Hospital Febrile Surveillance Participant Information and Consent Form**

**TUMAINI UNIVERSITY MAKUMIRA**

**KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE**

**P. O. Box 2240, Moshi   Tel. 027-27-53909**

**Brucellosis research in northern Tanzania**

**Hospital Patient-Participant Information Sheet**

## INTRODUCTION

You are being invited to take part in this research study because the examination at this health facility has shown that you have fever. Brucellosis is a disease that can cause fever and other symptoms and we are carrying out a research study on this disease. Before you decide if you want to take part in this study, it is important that you understand why the research is being done and what it will involve. Please read, or listen to, this information sheet and consent form carefully and take your time making your decision. As the study member discusses this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. We encourage you to talk with your family and friends before you decide to take part in this study. The nature of the study, length of time it will take, risks, and other important information about the study are listed below. If you agree to take part in this study, you will be asked to sign or add your thumbprint to this consent form. You will get a copy to keep.

## WHO IS DOING THIS RESEARCH?

This research will be conducted by experts in human and animal health from Tanzania and the United Kingdom. Dr. V Maro and Prof J. A. Crump from the Kilimanjaro Christian Medical Centre and Dr Gabriel Shirima from Nelson Mandela African Institute of Science and Technology, Tanzania; and Dr Jo Halliday from University of Glasgow will lead the field research for this project, other team members will also be involved. The research is funded by the Department for International Development (DFID) and five research councils in the UK.

## WHAT IS THE PURPOSE OF THIS STUDY?

In Tanzania, data collected by other studies has shown that brucellosis occurs in different types of animals (e.g. cattle, sheep and goats) and that brucellosis is an important cause of disease in people.

Brucellosis can be caused by several different types of *Brucella* bacteria. Each of these types of *Brucella* has different patterns of disease in animals. Information about which *Brucella* types are present in which animals in Tanzania is not well known. To develop plans for brucellosis control that are most likely to be effective and affordable, it is important to understand which animals are infected by which *Brucella* types, and which transmission routes are most important in transmitting brucellosis from animals to people. This three-year study will help to find out which *Brucella* types are found in different animal populations and which transmission routes are important for human disease. The information collected will help develop control strategies and will help improve *Brucella* identification in Tanzania.

## WHY HAVE I BEEN CHOSEN?

You have been invited to take part in this study because you have fever. Fever is one of the main signs of brucellosis. We would like to do some tests to find out if you do have brucellosis. We expect that about 360 people will take part in this study.

## DO I HAVE TO TAKE PART?

It is up to you to decide whether you do or do not to take part in this study. Participation is voluntary. If you agree to take part in this study, you will be asked to sign, or add your thumbprint to this consent form. You will get a copy to keep. If you decide to take part, you are free to stop at any time and you do not need to give a reason for stopping. If you decide to stop taking part in this study your contributions to the study would be removed from any study outputs produced after the date that you stop taking part.

## WHAT WILL HAPPEN TO ME IF I TAKE PART?

If you decide to take part in this study you will be asked to allow a member of the project team to take blood for brucellosis testing. Up to 50cc of blood will be collected. We will use the blood sample you give us to do tests for brucellosis and some other diseases including malaria. We will also use the sample to do more tests for brucellosis and other diseases at a later date. We will give you the results from some of these tests when they are available, if they tell us about your current illness. We will not give you the results of all the tests that are done with your samples. All of these tests are free. You will also be asked to respond to some questions about you, your household and your health. It will take a few hours for us to collect samples and ask you some questions, and we will complete this either today or possibly in the next few days if there is not time to complete our questionnaire today. We will also ask you to return to a follow-up clinic in 4-6 weeks time so that we can collect another blood sample for testing. Or, with your permission, the project team may visit you at your household to collect samples later. You can choose to stop taking part at any time without any cost.

## WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART IN THE STUDY?

The collection of blood samples may cause some pain, bleeding or bruising where the needle enters the body. A small blood clot may form where the needle enters the body or there may be swelling

in the area. In a small number of people lightheadedness and fainting can also happen when a blood sample is collected.

**WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART IN THIS STUDY?**
Direct benefits include free tests for brucellosis, other blood infections and malaria. The project team will also provide you with information about brucellosis. The results of the whole project will help to improve medical care and identification of diseases in Tanzania. We hope that the information collected through this study will be used to control brucellosis and reduce the impact that this disease has on human and animal health in Tanzania.

**WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?**
The samples we collect will be tested at Kilimanjaro Clinical Research Institute in Moshi. After this, more tests may be done at one of our partner institutions including the Animal and Plant Health Agency of the UK and University of Glasgow. We will store leftover samples after brucellosis testing so that they can be used for studies in the future. This may include studies of other diseases that cause fever. We do not plan to contact you with the results from tests done on stored samples. This is because the studies will most likely be performed many months or years after the samples were collected and the results would no longer be important for your treatment. The results from this study may be used by local and international institutions, responsible ministries, individuals and scientists.

**WILL MY INFORMATION BE KEPT CONFIDENTIAL?**
All information which is collected about you, or responses that you provide, during the course of the research study will be kept strictly confidential. You will not be identified by name in the study records shared outside KCMC or the University of Glasgow. If study results are given to other researchers, you will be identified by a code number, and any information about you will have your name and address removed so that you cannot be recognised from it.

**WHAT ABOUT COMPENSATION?**
If we ask you to return to the hospital for a second blood sample in 4-6 weeks time, we will provide you with money to cover the cost of your transport for this visit. No other compensation will be provided for your participation in this study.

**WHAT ARE MY RIGHTS AS A RESEARCH SUBJECT?**
Taking part in this study is completely voluntary. You may choose not to take part in this study. Your decision will not affect your ability to take part in other studies or result in the loss of any benefits you are entitled to.

**WHO HAS REVIEWED THIS STUDY?**
This study has been approved by the Ethics Committees of KCMC, the Tanzanian National Institute of Medical Research (NIMR) and the University of Glasgow.

**WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?**

For questions about this study contact: Dr Gabriel Shirima at 0763 973 003 or Dr Venance Maro at 0754 581 444. For questions about this study, to discuss problems, concerns or suggestions related to the study or to ask for information about this study, contact the Kilimanjaro Christian Medical Centre Ethics Committee at 027 275 3909. You can also contact the National Health Research Ethics Committee at 022 212 1400.

## <u>THANK YOU!</u>
### <u>On behalf of the project team we would like to thank you for agreeing to take part in this study</u>

**TUMAINI UNIVERSITY MAKUMIRA**

**KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE**

**P. O. Box 2240, Moshi   Tel. 027-27-53909**

Project Number:
Participant Unique Identification Number:

**CONSENT FORM**

**Brucellosis research in northern Tanzania - Participant (Adults)**

"The purpose of this study, what will happen to me and the risks and benefits have been explained to me. I have been allowed to ask questions, and I am satisfied with the answers I have received. I have been told who to contact if I have questions, to discuss problems, or suggestions related to the study, or to receive more information about the study. I have read (or had read) this information sheet and agree to take part in this research study, with the understanding that I may withdraw at any time. I have been told that I will be given a signed and dated copy of this consent form."

**Please initial or mark box**

I confirm that I have read (or had read) and understand the information sheet for
_____ participants, dated _____(version _____ ) for the above study
and have had the opportunity to ask questions.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.

I agree to take part in the above study.

_____        _____        _____
**Name of participant (print)**                          **Date**                       **Signature**

_____        _____        _____
**Study staff conducting consent (print)**            **Date**                       **Signature**

_____        _____        _____
**Witness' name (print) (As appropriate)**            **Date**                       **Signature**

(1 copy for subject; 1 copy for researcher)

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**TUMAINI UNIVERSITY MAKUMIRA**

**KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE**

**P. O. Box 2240, Moshi   Tel. 027-27-53909**

Project Number:
Participant Unique Identification Number:

### CONSENT FORM

**Brucellosis research in northern Tanzania – Participant (Minors)**

" The purpose of this study, what will happen to my child, and the risks and benefits have been explained to my child and me. I have been allowed to ask questions, and I am satisfied with the answers I have received. I have been told who to contact if I have questions, to discuss problems, or suggestions related to the study, or to receive more information about the study. I have read (or had read) this information sheet and agree for my child to take part in this research study, with the understanding that my child may withdraw at any time. I have been told that I will be given a signed and dated copy of this consent form."

**Please initial box**

I confirm that I have read (or had read) and understand the information sheet for _____ participants, dated _____(version _____ ) for the above study and have had the opportunity to ask questions.

I understand that my child's participation is voluntary and that we are free to withdraw at any time, without giving any reason or our legal rights being affected.

I agree for my child to take part in the above study.

Participant's name (print):      _____


_____     _____     _____
**Child Assent (print) (As appropriate)**      **Date**      **Signature**


_____     _____     _____
**Parent or Legal Guardian name (print)**      **Date**      **Signature**


_____     _____     _____
**Study staff conducting consent (print)**      **Date**      **Signature**


_____     _____     _____
**Witness' name (print) (As appropriate)**      **Date**      **Signature**

(1 copy for subject; 1 copy for researcher)

**Appendix 2: Febrile Participant Questionnaire**

# ZELS BRUCELLA - INDIVIDUAL QUESTIONNAIRE

## SECTION 1: INTERVIEW DETAILS

**1.1 Location ID**

Z B L - ☐ - ☐☐☐☐

**1.2 Enrollment Date (dd/mm/yyyy)**

☐☐ / ☐☐ / ☐☐☐☐

**1.3 Interviewer's initials**

*HH Q ID*

☐☐☐

**1.4 Individual ID**

Z B P - ☐☐☐☐

**1.5 Interview Date (dd/mm/yyyy)**

☐☐ / ☐☐ / ☐☐☐☐

*Review Date (dd/mm/yyyy)*

☐☐ / ☐☐ / ☐☐☐☐

*Reviewer's initials*

☐☐☐

**1.6 Language**

O Kiswahili   O English   O Maasai

**1.7 Primary informant**

O Self   O Parent/Guardian   O Relative   O Other

## SECTION 2: INDIVIDUAL DESCRIPTION

**2.1** Sex  **Jinsia**

O Male   O Female

**2.2** Date of birth (dd/mm/yyyy)  **Tarehe ya kuzaliwa**

☐☐ / ☐☐ / ☐☐☐☐

*If only the year of birth is known, record 01 for dd and 07 for mm.*
*If year of birth is known, ask question 2.4.*
*Kama ni mwaka wa kuzaliwa pekee unajulikana jaza 01 (dd) na 07 (mm)*
*Kama mwaka wa kuzaliwa unajulikana, uliza swali 2.4*

**2.3** Age Class

O 0-5 yr   O 6-12 yr   O 13-18 yr   O 19-34 yr   O 35-54 yr   O > 55 yr

**2.4** What is your tribe?  **Kabila lako?**

O Arusha          O Maasai
O Barabaig        O Pare
O Chagga          O Sambaa
O Iraqw           O Other (specify)

☐☐☐☐☐☐☐☐☐☐☐☐

*If Maasai or Arusha ask 2.5a/2.5b, otherwise proceed to 2.6*
*Kama ni Mmasai au Mwarusha uliza 2.5a/2.5b, vinginevyo nenda 2.6*

**2.5a.** What is your ageset (men)?
**Wewe ni rika gani (wanaume)?**

O Nyangulo
O Ilkiponi/Korrianga
O Ilkumunyak/ILandis (Ilkidotu)
O Ilkishumu/ Makaa (Irkishomo)
O ISeuri
O Ilnyankusi/Meshuki

**2.5b.** What is your ageset (women)?
(these are 'unofficial' but commonly used):
**Wewe ni rika gani (wanawake)?**

O Boda boda/Ingoipila: under 20yrs
O Njujulai: 20-30yrs
O Maharage (Intiamaragi)/N'gali: 30-36yrs
O Mosogiro: 37-46yrs
O Isusan: 47-56yrs
O Ingaimuk (Ingaimug): 56-65yrs
O Enderito: 65+yrs

**2.6** What is your marital status?  **Hali ya mahusiano ya ndoa?**

O Married (Nimeolewa/oa)
O Single (Sijaolewa/oa)
O Divorced/separated (Nimeachika/acha)
O Widowed (Miane)

**2.7** Arusha District/ **Wilaya ya Arusha**

O Arusha Rural     O Ngorongoro
O Arusha Urban     O Meru
O Longido          O Karatu
O Monduli          O Other

Other Region/ Mkoa mwingine

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Other District/ Wilaya nyingine

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

**2.8** Ward  **Kata**

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

**2.9** Village  **Kijiji**

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

**2.10** Sub-village  **Kitongoji**          *(leave blank if none)*

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

**2.11.** How long has your boma been in your current village?
**Ni kwa muda gani boma lako lipo katika kijiji hiki?**

O Years      O Months     O Days
**Miaka**     **Miezi**     **Siku**          ☐☐☐

**2.12** How many adults live in your boma?

☐☐ **Je, ni watu wazima wangapi wanaishi katika boma lako?**
*(age 18 years or older/ miaka 18 au zaidi)*

**2.13** How many children live in your boma?

☐☐ **Je, ni watoto wangapi wanaishi katika boma lako?**
*(age less than 18 years/ chini ya miaka 18)*

**2.14.** In the past 30 days, have you travelled outside your home region?
**Katika kipindi cha siku 30 zilizopita umesha safiri nje ya mkoa wako unaoishi?**

*If yes, provide details of the locations visited in the box below*
*Kama ndiyo, jaza katika kisanduku maeneo aliyo tembelea*

O Yes   O No

☐☐☐☐☐☐

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**2.15** How many years of education have you had?
**Umepata elimu (darasani) kwa miaka mingapi?**

○ No education (Sijasoma)

○ Primary (1-7 years) (Msingi)

○ Secondary (8-11 years) (Sekondari)

○ High school (12-13 years) (Sekondari ya juu)

○ University/college (Chuo kikuu/chuo)

**2.16** What are your main work/professional activities?
**Ajira yako kuu ni ipi?**

| | Primary occupation **Kazi ya kudumu** (Choose one) (Chagua moja) | Other occupation **Kazi ya ziada** (Choose many) (Chagua yote yanayohusika) |
|---|---|---|
| livestock attendant 1 **mchungi/mfugaji** | ○ | ○ |
| butcher/abattoir worker 2 **mchinjaji/mfanyakazi wa machinjioni** | ○ | ○ |
| wildlife worker 3 **mfanyakazi wa wanyamapori** | ○ | ○ |
| milk supplier 4 **msambazaji wa maziwa** | ○ | ○ |
| student 5 **mwanafunzi** | ○ | ○ |
| crafts person 6 **mhunzi** | ○ | ○ |
| veterinarian 7 **mganga wa mifugo** | ○ | ○ |
| housewife 8 **mama wa nyumbani** | ○ | ○ |
| office worker 9 **mfanyakazi wa ofisini** | ○ | ○ |
| healthcare worker 10 **mhudumu wa afya** | ○ | ○ |
| merchant/trader 11 **mjasiriamali/ mfanyabiashara** | ○ | ○ |
| teacher 12 **mwalimu** | ○ | ○ |
| driver 13 **dereva** | ○ | ○ |
| sewer worker 14 **wazibua vyoo** | ○ | ○ |
| guard/police 15 **askari/ polisi** | ○ | ○ |
| unemployed 16 **sijaajiriwa** | ○ | ○ |
| pre-working age 17 **bado hajafikia umri wa kufanya kazi** | ○ | ○ |
| other 18 **nyingineo** | ○ | |
| | | |

## SECTION 3: CURRENT AND RECENT ILLNESS

**3.1** During the past two weeks, have you had any of the following types of illness? (indicate all that apply)
**Katika wiki mbili zilizopita, umewahi kupata yoyote kati ya magonjwa yafuatayo? (ainisha yote yanayohusika)**

Diarrhoea/ **kuharisha**          ○ Yes ○ No

Respiratory illness/
**magonjwa ya kifua/ kupumua**          ○ Yes ○ No

Fever/ **homa**          ○ Yes ○ No

*diarrhoea: >= 3 loose stools within a 24 hours period*
*kuharisha: >= choo laini 3 ndani ya muda wa saa 24*
*respiratory illness: cough or difficulty breathing*
*magonjwa ya kifua/kupumua: kuhohoa au matatizo ya kupumua*
*fever: report of fever*

*If yes to fever in 3.1, ask questions 3.2 - 3.5*
*Kama jibu la homa ni ndio kwa swali 3.1, tafadhali uliza maswali 3.2 - 3.5*

**3.2** Is your fever continual or intermittent?
**Je, homa yako ni ya mfululizo au ya vipindi?**

○ Continual ○ Intermittent
   **mfululizo**   **vipindi**

**3.3** How long ago did the fever start?
**Je, ni muda gani tangu kuanza kwa homa?**

○ Days ○ Months ○ Years
   **siku**   **miezi**   **miaka**

[ ][ ]

**3.4** How long ago did your illness start?
**Je, ni muda gani ugonjwa wako ulikuanza?**

○ Days ○ Months ○ Years
   **siku**   **miezi**   **miaka**

[ ][ ]

**3.5** Have you had any of the following signs or symptoms of illness during this current illness?
**Umepata yoyote kati ya dalili zifuatazo za ugonjwa katika kipindi hiki cha kuugua kwako?**

*If not currently febrile, please ask for past 2 weeks and past 12 months only*
*Kama hana homa kwa sasa, uliza katika kipindi cha majuma mawili yaliyopita na miezi 12 iliyopita tu*

| | Current Illness | Past 2 weeks | Past 12 months |
|---|---|---|---|
| night sweats **jasho la usiku** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| fatigue **uchovu wa mwili** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| rigors **kutetemeka baridi** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| jaundice/yellow eyes **manjano/macho ya njano** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| joint pain **maumivu ya viungo** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| swollen joints **kuvimba kwa viungo** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| muscle pain **maumivu ya misuli** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| back pain **maumivu ya mgongo** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| headache **kuumwa kichwa** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| stiff neck **shingo kukakamaa** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| convulsions **degedege** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| conjunctival suffussion **macho kua mekundu** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| ear pain or drainage **maumivu ya sikio** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| sore throat **kuumwa koo** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| loss of appetite **kupoteza hamu ya kula** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| abdominal pain **maumivu ya tumbo** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| constipation **kuvimbiwa** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| bloody stool **choo chenye damu** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| vomiting **tapika** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| dysuria **unapata maumivu unapokojoa** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| swollen painful testicles (male only) **Kuvimba korodani/ makende (wanaume)** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| rash **upele** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |
| eschar **kovu** | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK | ◯ Yes ◯ No ◯ DK |

*For the next question:*
*Hospital participants should complete question.3.6a (and 3.6b if appropriate) but not 3.7a /3.7b.*
*Households participants should complete question 3.7a (and 3.7b if appropriate) but not 3.6a/ 3.6b*
*All participants should complete questions 3.8 onwards*

**3.6a** Have you sought care for this illness at another location prior to presentation at Endulen?
**Je, ulishatafuta tiba ya ugonjwa huu sehemu nyingine kabla ya Endulen?**
　　　◯ Yes ◯ No　*If yes, how many locations*
　　　　　　　　　　*Kama ndio, sehemu ngapi?*　☐☐

*If yes for question 3.6a ask 3.6b/ Kama ndio kwa sawali 3.6a, uliza 3.6b*

**3.6b** Which type of Healthcare facilities were visited?
**Ni aina gani ya vituo vya afya ulivyohudhuria?**
　◯ Pharmacy　　　**(Duka la dawa)**
　◯ Dispensary　　**(Zahanati)**
　◯ Traditional healer **(Mganga wa jadi)**
　◯ Health center　**(Kituo cha afya)**
　◯ Other Hospital　☐☐☐☐☐☐☐☐☐
　◯ Other
　　　　　　　　　☐☐☐☐☐☐☐☐☐

**For febrile participants in the household only. Kwa wagonjwa wa homa katika kaya tu.**

**3.7a** Have you sought care for your illness with a healthcare provider?
**Je, umesha tafuta tiba ya ugonjwa wako kwa mtoa huduma ya afya?**
　　　◯ Yes ◯ No　*If yes, how many*
　　　　　　　　　*Kama ndio, wangapi?*　☐☐

*If yes for question 3.7a ask 3.7b/ Kama ndio kwa sawali 3.7a, uliza 3.7b*

**3.7b** Which type of Healthcare facilities were visited?
**Ni aina gani ya vituo vya afya ulivyohudhuria?**
　◯ Pharmacy　　　**(Duka la dawa)**
　◯ Dispensary　　**(Zahanati)**
　◯ Traditional healer **(Mganga wa jadi)**
　◯ Health center　**(Kituo cha afya)**
　◯ Other Hospital　☐☐☐☐☐☐☐☐☐
　◯ Other
　　　　　　　　　☐☐☐☐☐☐☐☐☐

**3.8** Have you taken any medicines over the past 2 weeks?
**Umetumia dawa yoyote katika kipindi cha majuma 2 yaliyopita?**

　◯ Yes　◯ No　◯ DK　*If yes, what was the medication(s) for?*
　　　　　　　　　　*Kama ndio, dawa zilikua kwa ajili gani?*
**A.** ☐☐☐☐☐☐☐☐☐☐☐☐
**B.** ☐☐☐☐☐☐☐☐☐☐☐☐
**C.** ☐☐☐☐☐☐☐☐☐☐☐☐
**D.** ☐☐☐☐☐☐☐☐☐☐☐☐

*If yes, what was the medication(s) name(s)?*
*Kama ndio, dawa hizo zinaitwaje?*
**A.** ☐☐☐☐☐☐☐☐☐☐☐☐
**B.** ☐☐☐☐☐☐☐☐☐☐☐☐
**C.** ☐☐☐☐☐☐☐☐☐☐☐☐
**D.** ☐☐☐☐☐☐☐☐☐☐☐☐

☐☐☐☐☐☐☐

**3.9** Have you taken any other medications over the past 2 weeks?
**Umetumia dawa nyingine zozote katika kipindi cha majuma 2 yaliyopita?**

| | | | |
|---|---|---|---|
| Tuberculosis drugs/ **Dawa za kifua kikuu** | ○ Yes | ○ No | ○ DK |
| Analgesics/ **Dawa za kupunguza maumivu** | ○ Yes | ○ No | ○ DK |
| Antiretrovirals/ **Dawa za ARV** | ○ Yes | ○ No | ○ DK |
| Other/ **Nyinginezo** | ○ Yes | ○ No | ○ DK |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| | | | | | | | | | | |

## SECTION 4: PREGNANCY HISTORY (females only)

*We would now like to ask you some questions about your pregnancy history/*
***Tungependa sasa kukuuliza baadhi ya maswali kuhusu historia yako ya ujauzito***

**4.1** Are you comfortable answering questions about this?
**Je, unajisikia vizuri kujibu maswali kuhusiana na hili?**

○ Yes    ○ No

*If yes, proceed to next questions. If no, skip to SECTION 5*
*Kama ndio, endelea na maswali yafuatayo. Kama hapana, nenda SEHEMU ya 5*

**4.2** Have you started menstruating?
**Umeanza kupata siku za mwezi/ hedhi (umefikia kuvunja ungo)?**

○ Yes    ○ No

*If no, skip to SECTION 5/*
*Kama ni hapana nenda SEHEMU ya 5.*

**4.3** Have you ever been pregnant?
**Umewahi kupata ujauzito?**

○ Yes    ○ No

*If no, skip to SECTION 5./*
*Kama hapana, nenda SEHEMU ya 5*

**4.4** How many times have you been pregnant (including abortions, miscarriages, stillbirths and live births)?

**Ni mara ngapi umepata ujauzito (ikihusisha mimba zilizotolewa zilizoharibika, kuzaa watoto wafu na watoto hai)?** ☐☐

**4.5** How many live births have you had?
**Umejifungua mara ngapi watoto wakiwa hai?** ☐☐

**4.6** Have you ever had a miscarriage or stillbirth?
**Je, umewahi kupata mimba ikaharibika au kutoka yenyewe au kichanga kuzaliwa mfu?**

○ Yes    ○ No

*If yes, how many?*
*Kama ndio, mara ngapi?* ☐☐

**4.7** What was the outcome of your last pregnancy?
**Je, yapi yalikua matokeo ya ujauzito wako wa mwisho?**

☐☐☐☐☐☐☐

○ Miscarriage or spontaneous abortion (<28 weeks)
   **Mtoto kufia tumboni au mimba kuharibika (kabla ya wiki 28 za ujauzito)**
○ Fetal death or stillbirth (after 28 weeks)
   **Mtoto kufia tumboni/kuzaliwa mfu (baada ya wiki 28 za ujauzito)**
○ Live birth
   **Mtoto aliye hai**
○ NA- first pregnancy ongoing
   **Bado ni mjamzito**
○ Other **Nyingine**

☐☐☐☐☐☐☐☐☐☐☐☐☐☐

## SECTION 5: BRUCELLOSIS HISTORY

**5.1** Before we talked to you about this study, had you heard of a disease called brucellosis/ brucella?
**Kabla ya kuzungumza na wewe kuhusu huu utafiti, je uliwahi kusikia ugonjwa unaoitwa brucellosis/ brusela?**   ○ Yes ○ No

*If no to 5.1, skip to 5.9 / Kama hapana kwa swali 5.1, nenda swali la 5.9*

**5.2** Can you tell us what are the usual symptoms of brucellosis in people?
**Unaweza kutuambia dalili za mtu mwenye brucellosis?**

*Go through the list of symptoms/ signs and prompt the respondent to find out if they think each is associated with brucellosis. Record a Yes (Y) or No (N) response after prompting. record any additional reported signs or symptoms in the text box.*
*Pitia orodha ya dalili/ viashiria na muulize mshiriki kama dalili/ viashiria hivi vina uhusiano na brucellosis. Jaza jibu la Yes (Y) au No (N) baada ya kuuliza na jaza dalili zozote nyingine kwenye kisanduku mwishoni mwa jedwali.*

| | |
|---|---|
| I don't know/ **sijui** | ○ DK |
| malaise/ **uchovu** | ○ Yes ○ No |
| headache/ **maumivu ya kichwa** | ○ Yes ○ No |
| fever/ **homa** | ○ Yes ○ No |
| abdominal pain/ **maumivu ya tumbo** | ○ Yes ○ No |
| anorexia/ **kushindwa kula** | ○ Yes ○ No |
| joint pain/ **maumivu ya viungo** | ○ Yes ○ No |
| back pain/ **maumivu ya mgongo** | ○ Yes ○ No |
| sweats/ **kutokwa na jasho** | ○ Yes ○ No |
| chills/ **kutetemeka baridi** | ○ Yes ○ No |
| chest pain/ **maumivu ya kifua** | ○ Yes ○ No |
| muscle aches/ **kuumwa kwa misuli** | ○ Yes ○ No |
| cough/ **kukohoa** | ○ Yes ○ No |
| constipation/ **kufunga choo au kuvimbiwa** | ○ Yes ○ No |
| neck pain/ **maumivu ya shingo** | ○ Yes ○ No |
| diarrhoea/ **kuharisha** | ○ Yes ○ No |
| vomiting/ **kutapika** | ○ Yes ○ No |
| breathlessness/ **kupumua kwa shida** | ○ Yes ○ No |
| weight loss/ **kupungua kwa uzito** | ○ Yes ○ No |
| joint swelling/ **kuvimba kwa viungo** | ○ Yes ○ No |
| rash/ **upele** | ○ Yes ○ No |
| orchitis (in males)/ **kuvimba korodani** | ○ Yes ○ No |
| Other signs/ symptoms/ **Dalili/ viashiria vingine:** | |
| | |

**5.3** Have you ever been diagnosed with brucellosis?
**Je, ulishawahi kugundulika una brucellosis?**

          ○ Yes  ○ No

*If no to 5.3, skip to 5.8/ Kama hapana kwa  5.3, nenda 5.8*

**5.4** When was the diagnosis made? / **Je, uligundulika lini?**

Month/ **Mwezi**    Year/ **Mwaka**

[ ][ ] / [ ][ ][ ][ ]

**5.5** Where was the diagnosis made? / **Je, uligundulika wapi?**

[                    ]

**5.6** Did you receive treatment? /**Je, ulipatiwa matibabu?**

          ○ Yes  ○ No

*If no to 5.6, skip to 5.8/ Kama hapana kwa swali la 5.6, nenda 5.8*

**5.7** What was your treatment? / **Ulipatiwa tiba ipi?**

[                    ]

**5.8** Have any other members of your family ever been diagnosed with brucellosis?
**Je, kuna mtu yeyote katika kaya yako aliwahi kugundulika ana brucellosis?**

          ○ Yes  ○ No

*If yes, record who in the family, where and by who the diagnosis was made?*
*Kama ndio, jaza ni nani aliyegundulika katika familia na ni wapi na ni  nani aliye gundua?*

[                    ]

**If participant is febrile.**
**Kama mshiriki ana homa.**

**5.9** Do you know anyone in your boma or village who has current symptoms similar to yours?
**Je, unamjua mtu yeyote katika boma au kijiji chako mwenye dalili sawa na za kwako?**

          ○ Yes  ○ No

*If yes, fill out **"contact screening form"**./*
*Kama ndio, jaza fomu ya **"contact screening"***

Number of people
**Idadi ya watu**

| | | Number of people **Idadi ya watu** |
|---|---|---|
| Boma member **Mwanaboma** | ○ Yes ○ No ○ DK | [ ][ ] |
| Village member **Mwanakijiji** | ○ Yes ○ No ○ DK | [ ][ ] |

[ ][ ][ ][ ][ ][ ]   ZELS Brucella - Individual Questionnaire v1.0

## SECTION 6: FOOD

**6.1** In the past 30 days, have you consumed the following types of boiled or pasteurised dairy products?
**Katika siku 30 zilizopita, umetumia/ kula aina za mazao ya maziwa zilizochemshwa au za viwandani?**

*If yes, include how many days per week (in a typical week).*
*If no, ask if the dairy product has been consumed in the past 12 months*

*Kama  ndiyo, jaza ni siku ngapi katika juma (juma la kawaida)*
*Kama hapana, uliza kama aina hiyo ya mazao ya maziwa alitumia katika kipindi cha miezi 12 iliyopita*

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| Milk **Maziwa** | ○ Yes ○ No | [ ] | ○ Yes ○ No |
| Yogurt **Maziwa mtindi** | ○ Yes ○ No | [ ] | ○ Yes ○ No |
| Cheese **Jibini** | ○ Yes ○ No | [ ] | ○ Yes ○ No |
| Butter **Siagi** | ○ Yes ○ No | [ ] | ○ Yes ○ No |
| Cream **Mafuta ya maziwa** | ○ Yes ○ No | [ ] | ○ Yes ○ No |
| Other food (e.g. uji, ndizi) prepared by adding dairy products before or during cooking **Vyakula vingine (mfano uji, ndizi)  kwa kuviongezea mazao ya maziwa kabla au wakati wakupikwa** | ○ Yes ○ No | [ ] | ○ Yes ○ No |
| Other food (e.g. uji, ndizi) prepared by adding pasteurised or boiled dairy products after cooking **Vyakula vingine (mfano uji, ndizi) kwa kuviongezea mazao ya maziwa yaliyochemshwa baada ya kupika** | ○ Yes ○ No | [ ] | ○ Yes ○ No |
| Other products made from  boiled or pasteurised dairy products **Mazao mengine yatokanayo na  maziwa ya viwandani au maziwa yaliyochemshwa** | ○ Yes ○ No | [ ] | ○ Yes ○ No |
| | | [ ][ ][ ][ ][ ][ ] | |
| cheese, butter, cream or yogurt but unsure whether milk raw or boiled/  pasteurised **Jibini, siagi, mafuta au mgando/mtindi lakini hakuna uhakika kama maziwa yalikuwa mabichi, yaliyochemshwa au ya kiwandani** | ○ Yes ○ No | [ ] | ○ Yes ○ No |

**6.2** Which animals did the boiled or pasteurised milk products come from? (Choose all that apply and prompt all options)
**Je, ni kutoka kwa wanyama wepi maziwa yaliyochemshwa au bidhaa za maziwa ya kiwandani yamepatikana?**
(Chagua yote yanayohusika)

| | |
|---|---|
| cow/ **ng'ombe wako** | ○ Yes ○ No |
| goat/ **mbuzi wako** | ○ Yes ○ No |
| sheep/ **kondoo wako** | ○ Yes ○ No |
| other animal/ **wengine** | ○ Yes ○ No |
| [ ][ ][ ][ ][ ][ ][ ][ ] | |

**6.3** In the past 30 days, have you consumed any of the following types of raw meat or offal or raw animal blood?
**Katika kipindi cha siku 30 zilizopita, je wewe umetumia/kula yoyote kati ya aina ya nyama mbichi au nyama za ndani, au damu mbichi ya mnyama?**

*If yes, include how many days per week (in a typical week).*
*If no, ask if the meat/offal/blood has been consumed in the past 12 months.*

*Kama ndio, uliza ni siku ngapi katika juma (juma la kawaida)*
*Kama hapana, uliza kama nyama mbichi/nyama za ndani/ damu mbichi ilitumika miezi 12 iliyopita*

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| raw cow blood<br>**damu mbichi ya ng'ombe** | O Yes<br>O No | ☐ | O Yes<br>O No |
| raw goat blood<br>**damu mbichi ya mbuzi** | O Yes<br>O No | ☐ | O Yes<br>O No |
| raw sheep blood<br>**damu mbichi ya kondoo** | O Yes<br>O No | ☐ | O Yes<br>O No |
| raw blood from other animal<br>**damu mbichi ya mnyama mwingine** | O Yes<br>O No | ☐ | O Yes<br>O No |
| | ☐☐☐☐☐☐☐☐☐☐ | | |
| raw meat or offal from cow<br>**nyama mbichi au nyama za ndani kutoka kwa ng'ombe** | O Yes<br>O No | ☐ | O Yes<br>O No |
| raw meat or offal from goat<br>**nyama mbichi au za ndani kutoka kwa mbuzi** | O Yes<br>O No | ☐ | O Yes<br>O No |
| raw meat or offal from sheep<br>**nyama mbichi au za ndani kutoka kwa kondoo** | O Yes<br>O No | ☐ | O Yes<br>O No |
| raw meat or offal from another animal<br>**nyama mbichi au za ndani kutoka kwa mnyama mwingine** | O Yes<br>O No | ☐ | O Yes<br>O No |
| | ☐☐☐☐☐☐☐☐☐☐ | | |

**6.4** In the past 30 days have you consumed soup with blood?
**Je, katika kipindi cha siku 30 zilizopita uliwahi kunywa supu yenye damu (kisusio)?**　　O Yes　O No

*If yes, blood from which animal?*
*Kama ndio, ni damu kutoka kwa mnyama yupi?*

| | |
|---|---|
| Cow/ **Ng'ombe** | O Yes O No |
| Goat/ **Mbuzi** | O Yes O No |
| Sheep/ **Kondoo** | O Yes O No |
| Other/ **Wengine** | O Yes O No |
| ☐☐☐☐☐☐☐☐☐☐ | |

**6.5** In the past 30 days have you consumed blood mixed with milk?
**Je, katika kipindi cha siku 30 zilizopita uliwahi kunywa maziwa yaliyochanganywa na damu (mlaso)?**
　　　　　　　　　　　　　　　　　　O Yes　O No

*If yes, blood from which animal?*
*Kama ndio, ni damu kutoka kwa mnyama yupi?*

| | |
|---|---|
| Cow/ **Ng'ombe** | O Yes O No |
| Goat/ **Mbuzi** | O Yes O No |
| Sheep/ **Kondoo** | O Yes O No |
| Other/ **Wengine** | O Yes O No |
| ☐☐☐☐☐☐☐☐☐☐ | |

*If yes, milk from which animal?*
*Kama ndio, ni maziwa kutoka kwa mnyama yupi?*

| | |
|---|---|
| Cow/ **Ng'ombe** | O Yes O No |
| Goat/ **Mbuzi** | O Yes O No |
| Sheep/ **Kondoo** | O Yes O No |
| Other/ **Wengine** | O Yes O No |
| ☐☐☐☐☐☐☐☐☐☐ | |

**6.6** In the past 12 months, have you consumed meat or offal from an aborted animal or the placenta of an aborted animal?
**Katika miezi 12 iliyopita, umetumia/kula nyama au nyama za ndani kutoka kwa kichanga cha mnyama au kondo la nyuma?**
　　　　　　　　　　　　　　　　　O Yes　　O No

*If no, skip to 6.7*
*If yes, complete table below. If activity was performed in past 30 days, include how many days per week (in a typical week).*

*Kama hapana, nenda swali 6.7*
*Kama ndio, kamilisha jedwali lifuatalo. Kama shughuli ilifanyika katika siku 30 zilizopita jaza ni siku ngapi katika juma (juma la kawaida)*

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| Cow / N**g'ombe** | O Yes O No | ☐ | O Yes O No |
| Goat/ **Mbuzi** | O Yes O No | ☐ | O Yes O No |
| Sheep/ **Kondoo** | O Yes O No | ☐ | O Yes O No |
| Another animal<br>**Mnyama mwingine** | O Yes O No | ☐ | O Yes O No |
| ☐☐☐☐☐☐☐☐☐☐☐ | | | |

**6.7** Was the meat or offal raw?
**Je, nyama/ nyama za ndani zilikua mbichi?**　　O Yes O No

**6.8** In the past 30 days, have you consumed the following types of raw dairy products?

**Katika siku 30 zilizopita, umetumia/ kula aina za mazao ya maziwa mabichi zifuatazo?**

*If yes, include how many days per week (in a typical week).*
*If no, ask if the raw dairy product has been consumed in the past 12 months*

*Kama ndiyo, jaza ni siku ngapi katika juma (juma la kawaida)*
*Kama hapana, uliza kama aina hiyo ya mazao ya maziwa mabichi yalitumika katika kipindi cha miezi 12 iliyopita*

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| Milk **Maziwa** | ○ Yes ○ No | | ○ Yes ○ No |
| Yogurt **Maziwa mtindi** | ○ Yes ○ No | | ○ Yes ○ No |
| Cheese **Jibini** | ○ Yes ○ No | | ○ Yes ○ No |
| Butter **Siagi** | ○ Yes ○ No | | ○ Yes ○ No |
| Cream **Mafuta ya maziwa** | ○ Yes ○ No | | ○ Yes ○ No |
| Other food (e.g. uji, ndizi) prepared by adding raw dairy products before or during cooking **Vyakula vingine (mfano uji, ndizi) kwa kuongeza mazao ya maziwa mabichi kabla au wakati unapika** | ○ Yes ○ No | | ○ Yes ○ No |
| Other food (e.g. uji, ndizi) prepared by adding raw dairy products after cooking **Vyakula vingine (mfano uji, ndizi) kwa kuongeza mazao ya maziwa mabichi baada ya kupika** | ○ Yes ○ No | | ○ Yes ○ No |
| Other products made from other raw dairy products **Mazao mengine yatokanayo katika mazao mengine ya maziwa mabichi** | ○ Yes ○ No | | ○ Yes ○ No |

[ ][ ][ ][ ][ ][ ][ ][ ][ ]

**6.9** If you do not tend to drink raw milk, or consume it in other dairy or food products, are there any particular circumstances where you might consume raw milk or it's products?

**Kama huna nia ya kunywa maziwa mabichi au kuyatumia katika mazao yoyote ya maziwa au vyakula, je kuna mazingira yoyote huenda yakasababisha kunywa maziwa mabichi au mazao yake?**

○ Yes    ○ No    *If yes, under what circumstances?*
*Kama ndio, ni katika mazingira gani?*

[ ]

## SECTION 7:ANIMAL RELATED ACTIVITIES

**7.1** Have you milked any animals in the past 12 months?
**Je wewe umekamua wanyama wowote katika kipindi cha miezi 12 iliyopita?**
○ Yes ○ No

*If no, skip to 7.2*
*If yes, complete the following table. If activity was performed in past 30 days, include how many days per week (in a typical week).*

*Kama hapana nenda swali 7.2*
*Kama ndio, kamilisha jedwali lifuatalo. Kama shughuli ilifanyika katika siku 30 zilizopita jaza ni siku ngapi katika juma (juma la kawaida)*

[ ][ ][ ][ ][ ][ ][ ]

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| cattle **ng'ombe** | ○ Yes ○ No | | ○ Yes ○ No |
| goats **mbuzi** | ○ Yes ○ No | | ○ Yes ○ No |
| sheep **kondoo** | ○ Yes ○ No | | ○ Yes ○ No |
| another animal **wengineo** | ○ Yes ○ No | | ○ Yes ○ No |

[ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

**7.2** In the past 12 months, have you slept in the same room or enclosure as any animals?
**Katika miezi 12 iliyopita, umelala usiku kucha katika chumba kimoja au sehemu moja na wanyama wowote?**
○ Yes ○ No

*If no, skip to 7.3*
*If yes, complete table below. If activity was performed in past 30 days, include how many days per week (in a typical week).*
*Kama hapana nenda swali 7.3*
*Kama ndio, kamilisha jedwali lifuatalo. Kama shughuli ilifanyika katika siku 30 zilizopita jaza ni siku ngapi katika juma (juma la kawaida)*

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| cattle **ng'ombe** | ○ Yes ○ No | | ○ Yes ○ No |
| goats **mbuzi** | ○ Yes ○ No | | ○ Yes ○ No |
| sheep **kondoo** | ○ Yes ○ No | | ○ Yes ○ No |
| another animal **wengineo** | ○ Yes ○ No | | ○ Yes ○ No |

[ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

**7.3** In the past 12 months have you handled the waste (manure) of any animals, including during building construction, cleaning animal pens, use as fertiliser etc.?
**Katika kipindi cha miezi 12 iliyopita umeshashika samadi ya mnyama yeyote ikiwemo katika ujenzi, usafi wa zizi, matumizi ya mbolea n.k?**
○ Yes    ○ No

*If no, skip to7.4*
*If yes, complete table below. If activity was performed in past 30 days, include how many days per week (in a typical week).*
*Kama hapana nenda swali 7.4*
*Kama ndio, kamilisha jedwali lifuatalo. Kama shughuli ilifanyika katika siku 30 zilizopita jaza ni siku ngapi katika juma (juma la kawaida)*

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| cattle **ng'ombe** | ○ Yes ○ No | | ○ Yes ○ No |
| goats **mbuzi** | ○ Yes ○ No | | ○ Yes ○ No |
| sheep **kondoo** | ○ Yes ○ No | | ○ Yes ○ No |
| another animal **wengineo** | ○ Yes ○ No | | ○ Yes ○ No |

[ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

**7.4** Have you herded or used any animals for herding in the past 12 months?
**Je, wewe umechunga au kumtumia yoyote kati ya wanyama kwa ajili ya kuchungia katika miezi 12 iliyopita?**
○ Yes   ○ No

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*If no, skip to 7.5*
*If yes, complete table below. If activity was performed in past 30 days, include how many days per week (in a typical week).*
*Kama hapana nenda swali 7.5*
*Kama ndio, kamilisha jedwali lifuatalo. Kama shughuli ilifanyika katika siku 30 zilizopita jaza ni siku ngapi katika juma (juma la kawaida)*

|  | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| cattle **ng'ombe** | ○ Yes ○ No |  | ○ Yes ○ No |
| goats **mbuzi** | ○ Yes ○ No |  | ○ Yes ○ No |
| sheep **kondoo** | ○ Yes ○ No |  | ○ Yes ○ No |
| dogs **mbwa** | ○ Yes ○ No |  | ○ Yes ○ No |
| another animal **mnyama mwingine** | ○ Yes ○ No |  | ○ Yes ○ No |

**7.5** Have you assisted with the birthing of any animals in the past 12 months?
**Je, ulisaidia kuzalisha mnyama yeyote katika miezi 12 iliyopita?**

○ Yes   ○ No

*If yes, number of animals in past 30 days*
*If no, ask if they have assisted with birthing of any of these animals in the past 12 months*
*Kama ndio, idadi ya wanyama katika kipindi cha siku 30 zilizopita*
*Kama hapana, uliza kama walisaidia kuzalisha wanyama wowote katika miezi 12 iliyopita*

|  | Past 30 days | Number of animals | Past 12 months |
|---|---|---|---|
| cattle **ng'ombe** | ○ Yes ○ No |  | ○ Yes ○ No |
| goats **mbuzi** | ○ Yes ○ No |  | ○ Yes ○ No |
| sheep **kondoo** | ○ Yes ○ No |  | ○ Yes ○ No |
| donkeys **punda** | ○ Yes ○ No |  | ○ Yes ○ No |
| another animal **mnyama mwingine** | ○ Yes ○ No |  | ○ Yes ○ No |

**7.6** Have you handled/had contact with any placental or birth material of any animals in the past 12 months?
**Je, umewahi kushika/kugusa kondo la nyuma au vitu vya uzazi vya mnyama yeyote katika miezi 12 iliyopita?**

○ Yes   ○ No

*If no, skip to 7.7 If yes, complete table below.*
*Kama hapana, nenda swali 7.7*
*Kama ndio,kamilisha jedwali lifuatalo*

|  | Past 30 days | Number of animals | Past 12 months |
|---|---|---|---|
| cattle **ng'ombe** | ○ Yes ○ No |  | ○ Yes ○ No |
| goats **mbuzi** | ○ Yes ○ No |  | ○ Yes ○ No |
| sheep **kondoo** | ○ Yes ○ No |  | ○ Yes ○ No |
| dogs **mbwa** | ○ Yes ○ No |  | ○ Yes ○ No |
| another animal **mnyama mwingine** | ○ Yes ○ No |  | ○ Yes ○ No |

**7.7** Have you handled/had contact with any aborted birth products from any animals in the past 12 months, including dead young/ offspring, animal fluid, placenta or blood?
**Je, umewahi kushika/ kugusa vitu vyovyote vya mimba iliyotoka/ haribika ikiwemo kichanga mfu, maji maji ya mnyama, kondo la nyuma au damu kutoka kwa mnyama yoyote katika miezi 12 iliyopita?**

○ Yes   ○ No

*If no, skip to 7.8 .If yes, complete table below.*
*Kama hapana, nenda swali 7.8 Kama ndio, kamilisha jedwali lifuatalo*

|  | Past 30 days | Past 12 months |
|---|---|---|
| cattle/ **ng'ombe** | ○ Yes ○ No | ○ Yes ○ No |
| goats/ **mbuzi** | ○ Yes ○ No | ○ Yes ○ No |
| sheep/ **kondoo** | ○ Yes ○ No | ○ Yes ○ No |
| another animal/ **wengineo** | ○ Yes ○ No | ○ Yes ○ No |

**7.8** Have you slaughthered or butchered (or assisted in butchering) any livestock or domestic animals in the past 12 months?
**Je wewe ulichinja au kukatakata (au kusaidia kuchinja au kukatakata) yeyote kati ya mifugo au wanyama wanaofugwa katika miezi 12 iliyopita?**

○ Yes   ○ No

*If no, skip to 7.9. If yes, complete table below.*
*Kama hapana, nenda swali 7.9 Kama ndio, kamilisha jedwali lifuatalo*

|  | Past 30 days | Past 12 months |
|---|---|---|
| cattle/ **ng'ombe** | ○ Yes ○ No | ○ Yes ○ No |
| goats/ **mbuzi** | ○ Yes ○ No | ○ Yes ○ No |
| sheep/ **kondoo** | ○ Yes ○ No | ○ Yes ○ No |
| pigs/ **nguruwe** | ○ Yes ○ No | ○ Yes ○ No |
| another animal/ **mwingine** | ○ Yes ○ No | ○ Yes ○ No |

**7.9** Have you handled/had contact with the carcass/ carcasses of any livestock or domestic animals in the past 12 months?
**Je, umewahi kushika/ kugusa mzoga/ mizoga ya mifugo au wanyama wanaofugwa katika kipindi cha miezi 12 iliyopita?**

○ Yes   ○ No

*If no, skip to 7.10 .If yes, complete table below.*
*Kama hapana, nenda swali 7.10. Kama ndio, kamilisha jedwali lifuatalo*

|  | Past 30 days | Number of animals | Past 12 months |
|---|---|---|---|
| cattle **ng'ombe** | ○ Yes ○ No |  | ○ Yes ○ No |
| goats **mbuzi** | ○ Yes ○ No |  | ○ Yes ○ No |
| sheep **kondoo** | ○ Yes ○ No |  | ○ Yes ○ No |
| pigs **nguruwe** | ○ Yes ○ No |  | ○ Yes ○ No |
| dogs **mbwa** | ○ Yes ○ No |  | ○ Yes ○ No |
| donkeys **punda** | ○ Yes ○ No |  | ○ Yes ○ No |
| another animal **mnyama mwingine** | ○ Yes ○ No |  | ○ Yes ○ No |

**7.10** Have you handled/had contact with the carcass/ carcasses of any wild animals in the past 12 months?
**Je, umewahi kushika/ kugusa mzoga/ mizoga ya wanyama wa wowote wa mwituni katika kipindi cha miezi 12 iliyopita?** ○ Yes ○ No

*If no, skip to 7.11 .If yes, complete table below.*
*Kama hapana, nenda swali 7.11.Kama ndio, kamilisha jedwali lifuatalo*

| | Past 30 days | Number of animals | Past 12 months |
|---|---|---|---|
| zebra **pundamilia** | ○ Yes ○ No | | ○ Yes ○ No |
| wildebeest **nyumbu** | ○ Yes ○ No | | ○ Yes ○ No |
| buffalo **nyati** | ○ Yes ○ No | | ○ Yes ○ No |
| antelope **swala** | ○ Yes ○ No | | ○ Yes ○ No |
| another animal **wengineo** | ○ Yes ○ No | | ○ Yes ○ No |
| | | | |

**7.11** Have you handled/prepared raw animal blood, meat or offal in the past 12 months?
**Katika kipindi cha miezi 12 iliyopita umeshika/ andaa damu mbichi, nyama au nyama za ndani za wanyama?** ○ Yes ○ No

*If yes, complete the following table.If no, skip to next question.*
*Kama ndio, kamilisha jedwali lifuatalo. Kama hapana nenda swali linalofuata*

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| Cow **Ng'ombe** | ○ Yes ○ No | | ○ Yes ○ No |
| Goat **Mbuzi** | ○ Yes ○ No | | ○ Yes ○ No |
| Sheep **Kondoo** | ○ Yes ○ No | | ○ Yes ○ No |
| Another animal **Mnyama mwingine** | ○ Yes ○ No | | ○ Yes ○ No |
| | | | |

**7.12** Have you handled or prepared animal hides from any animal in the past 12 months?
**Katika kipindi cha miezi 12 iliyopita umeshika au kuandaa ngozi za mnyama au wanyama wowote?** ○ Yes ○ No

*If yes, complete table below. If no, skip to next question.*
*Kama ndio, kamilisha jedwali lifuatalo. Kama hapana, uliza swali linalofuata.*

| | Past 30 days | Number of days (1-7) | Past 12 months |
|---|---|---|---|
| cow **ng'ombe** | ○ Yes ○ No | | ○ Yes ○ No |
| goat **mbuzi** | ○ Yes ○ No | | ○ Yes ○ No |
| sheep **kondoo** | ○ Yes ○ No | | ○ Yes ○ No |
| another animal **mwingineo** | ○ Yes ○ No | | ○ Yes ○ No |
| | | | |

**7.13** Have any of your family's livestock kept at your boma, aborted or delivered still-born offspring in the last 12 months?
**Kuna mfugo yoyote (ng'ombe, kondoo au mbuzi) anayefugwa katika boma hili,ametoa/ameharibu mimba au amezaa mtoto mfu katika miezi 12 iliopita?**

*If yes, indicate the number of abortions/ stillbirths in the last 12 months.*
*Record NA if that species is not kept at this boma*
*Kama ndio, ainisha idadi ya mimba zilizoharibika/ watoto waliozaliwa wafu katika miezi 12 iliopita.*
*Jaza NA kama mnyama hafugwi katika boma hili*

Cattle **(Ng'ombe)**
○ Yes ○ No ○ DK ○ NA    *If yes how many Kama ndio, wangapi?*

Sheep **(Kondoo)**
○ Yes ○ No ○ DK ○ NA    *If yes how many Kama ndio, wangapi?*

Goats **(Mbuzi)**
○ Yes ○ No ○ DK ○ NA    *If yes how many Kama ndio, wangapi?*

Other Animal **(Mnyama mwingine)**
○ Yes ○ No ○ DK ○ NA    *If yes how many Kama ndio, wangapi?*

**7.14** Have any of your family's animals died in the past 30 days?
*(do not include animals intentionally slaughtered)*

**Kuna yoyote kati ya mifugo ya familia yako imekufa katika kipindi cha siku 30 zilizopita?**
*(usihusishe wanyama waliochinjwa)* ○ Yes ○ No ○ DK

*If yes, which animals and how many?*
*Kama ndio, wanyama wepi na wangapi?*

| | | Number adult **Idadi ya wanyama wakubwa** | Number young **Idadi ya wanyama watoto** |
|---|---|---|---|
| Cow **Ng'ombe** | ○ Yes ○ No ○ DK | | |
| Goat **Mbuzi** | ○ Yes ○ No ○ DK | | |
| Sheep **Kondoo** | ○ Yes ○ No ○ DK | | |
| Donkeys **Punda** | ○ Yes ○ No ○ DK | | |
| Other specify/ **Wengineo** | ○ Yes ○ No ○ DK | | |
| | | | |

*If yes to 7.14, proceed to 7.15. If no, skip to SECTION 8*
*Kama ndio kwa swali 7.14, uliza 7.15. Kama hapana endelea SEHEMU ya 8*

**7.15** Can you list the causes of death for your animals?
**Unaweza kuorodhesha sababu ya vifo kwa wanyama wako?**

<br>

## SECTION 8: WATER USAGE

**8.1** What is your primary source of drinking water in the dry & wet seasons?
**Chanzo kikuu cha maji ya kunywa kwa wanakaya katika kipindi cha ukame na masika ni kipi?**

| | Dry season primary source (select one) **Kiangazi, chanzo kikuu (chagua moja tu)** | Dry season, other sources **Msimu wa ukame, vyanzo vingine** | Wet season primary source **Masika, chanzo kikuu (chagua moja tu)** | Wet season, other sources **Msimu wa mvua, vyanzo vingine** |
|---|---|---|---|---|
| Piped water into the home **Maji ya bomba nyumbani** | O | O | O | O |
| Piped water near the home **Bomba ndani karibu na boma** | O | O | O | O |
| Public/ communal well or standpipe **Kisima cha umma, bomba ya umma** | O | O | O | O |
| River or creek (moving water) directly **Moja kwa moja kutoka katika mto au mfereji (maji yanayotembea)** | O | O | O | O |
| Lake, pond, dam (standing water) directly **Moja kwa moja kutoka katika ziwa, dimbwi, au bwawa (maji yaliyosimama)** | O | O | O | O |
| Private well or pump **Kisima au pampu binafsi** | O | O | O | O |
| From a spring **Moja kwa moja kutoka katika chemchem** | O | O | O | O |
| Rainwater **Maji ya mvua** | O | O | O | O |
| Tanker truck **Gari la kubebea maji (boza)** | O | O | O | O |
| Other (specify) **Vinginevyo (ainisha)** | O | O | O | O |
| ▯▯▯▯▯▯▯▯▯▯▯▯▯ | | | | |

**8.2** Is your drinking water treated
(by filtering, boiling, chlorinating, straining, etc.)?
**Huwa una tibu maji ya kunywa (kwa kuchuja, kuchemsha, kuweka dawa ya klorine, kuchuja kwa nguo, nk)?**

O Yes    O No

*If yes, how frequently do you treat your household drinking water?*
*Kama ndio, mara ngapi una tibu maji ya kunywa katika kaya yako?*

O Always **Kila wakati**    O Sometimes **Muda kwa muda**    O Never **Hatujafanya chochote**    O DK **Sijui**

*If always or sometimes, answer 8.3, otherwise skip to SECTION 9*
*Kama ni "kila wakati" au "muda kwa muda", jibu swali 8.3, vinginevyo nenda SEHEMU ya 9.*

**8.3** How do you treat it? (choose all that apply)
**Kama ndio, unatibu vipi ? (chagua yote yanayohusika)**

O Boiling/ **Kuchemsha**

O Sedimentation and decant/ **Kuacha kwa muda yatwae/uchafu uende chini**

O Other, specify/ **Nyinginezo, ainisha**

O Strain it through a cloth/ **Kuchuja kwa nguo**

O Solar disinfection/ **Kuweka juani**

O Adding disinfectant, such as chlorine or bleach **Kuweka dawa kama klorine/ bleach/water guard**

O Filtering/ **Kuchuja**

▯▯▯▯▯▯▯▯▯▯▯▯▯▯

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## SECTION 9: HOME

**9.1** What type of toilet system do members of your home normally use? (choose only one)
**Ni aina gani ya mfumo wa choo ambao kwa kawaida unatumika na wakazi wa nyumbani kwako? (chagua moja tu)**

○ Flush or pour toilet with septic tank, including squat toilet
**Choo cha kuchuchumaa, cha kumwaga maji na mfumo wa shimo la maji taka**

○ Flush or pour toilet connected to sewer pipe
**Choo cha maji kilichounganishwa na bomba la maji taka**

○ Pit latrine with covering slab
**Choo cha shimo kilichosakafiwa**

○ Pit latrine without covering slab
**Choo cha shimo ambacho hakijasakafiwa (kisichosakafiwa)**

○ Ventilated improved pit latrine (VIP)
**Choo cha shimo bora chenye bomba la kutoa hewa chafu (VIP)**

○ Bucket or plastic bags
**Ndoo au mifuko**

○ No facilites or field or bush
**Hakuna choo wala kwenda porini**

**9.2** Do you have electricity in your home?
**Una umeme wowote nyumbani kwako?**   ○ Yes   ○ No

*If yes, ask question 9.3.  If no, skip to 9.4*
*Kama ndio, uliza swali 9.3. Kama hapana uliza swali 9.4*

**9.3** What kind of electricity do you have?
**Unatumia umeme wa aina gani?**

○ Grid (Gridi)
○ Solar (Solar)
○ Generator (Jenerata)
○ Other (Mwingineo)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|

**9.4** What type of energy sources are used for cooking in your home? *(primary and secondary sources)*
**Aina gani kuu (ya msingi) ya nishati inatumika kwa kupikia nyumbani kwako?**

| | Primary source (choose one) **Chanzo cha kudumu (Chagua moja)** | Other source (choose all that apply) **Vyanzo vingine (chagua yote yanoyohusika)** |
|---|---|---|
| Electricity/ **Umeme** | ○ | ○ |
| Gas/ **Gesi** | ○ | ○ |
| Kerosene/ **Mafuta taa** | ○ | ○ |
| Cow dung/ **Kinyesi cha ng'ombe** | ○ | ○ |
| Firewood/ **Kuni** | ○ | ○ |
| Charcoal/ **Mkaa** | ○ | ○ |
| Other/ **Nyinginezo** | ○ | ○ |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|

**9.5** Do the members of this home (all combined) own any of the following items? (choose all that apply)
**Je, wakazi wa nyumba hii (wote pamoja) wanamiliki chochote kati ya vitu vifuatavyo? (chagua yote yanayohusika)**

*Please enter 00 in the Number of units field for items that are not owned at this home*
*Tafadhali jaza 00 katika sehemu ya idadi ya namba kwenye sehemu ambayo vitu hivyo havimilikiwi katika nyumba hii.*

|  | Number of Unit **Ngapi?** | If respondent is Female: **Kama mhojiwa ni mwanamke:** Are any of these items yours personally? **Vitu vyote vyako?** |
|---|---|---|
| Ox plough (**Jembe la ng'ombe**) |  | ○ Yes ○ No |
| Ox cart (**Mkokoteni wa ng'ombe**) |  | ○ Yes ○ No |
| Bicycle (**Baiskeli**) |  | ○ Yes ○ No |
| Motorbike (**Pikipiki**) |  | ○ Yes ○ No |
| Car (**Gari**) |  | ○ Yes ○ No |
| Tractor (**Trekta**) |  | ○ Yes ○ No |
| Mobile phone(**Simu ya mkononi**) |  | ○ Yes ○ No |
| Radio (**Redio**) |  | ○ Yes ○ No |
| Television (**Luninga**) |  | ○ Yes ○ No |
| Sofa (**Makochi**) |  | ○ Yes ○ No |
| Bed net (**Chandarua**) |  | ○ Yes ○ No |
| Refrigerator (**Jokofu au friji**) |  | ○ Yes ○ No |
| A Business (**Biashara**) |  | ○ Yes ○ No |

**9.6** How many structures/ buildings including livestock bomas and homes in total are in your boma?
**Kuna idadi gani ya mifumo/ majengo, ikiwemo maboma ya mifugo pamoja na idadi ya nyumba kwa ujumla katika boma lako?**

| | | |
|---|---|---|

Comments/ **Maelezo:**

**Appendix 3: Household Surveillance Participant Information and Consent Form**

**TUMAINI UNIVERSITY MAKUMIRA**

**KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE**

**P. O. Box 2240, Moshi   Tel. 027-27-53909**

**Brucellosis research in northern Tanzania**
**Patient contact - Participant Information Sheet**

## INTRODUCTION

You are being invited to take part in this research study because a member of your household or village has been shown to have fever or a recent history of fever. Brucellosis is a disease that can cause fever and other symptoms and we are carrying out a research study on this disease. Before you decide if you want to take part in this study, it is important that you understand why the research is being done and what it will involve. Please read, or listen to, this information sheet and consent form carefully and take your time making your decision. As the study member discusses this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. We encourage you to talk with your family and friends before you decide to take part in this study. The nature of the study, length of time it will take, risks and other important information about the study are listed below. If you agree to take part in this study, you will be asked to sign or add your thumbprint to this consent form. You will get a copy to keep.

## WHO IS DOING THIS RESEARCH?

This research will be conducted by experts in human and animal health from Tanzania and the United Kingdom. Dr. V Maro and Prof J. A. Crump from the Kilimanjaro Christian Medical Centre; Dr Gabriel Shirima from Nelson Mandela African Institute of Science and Technology; and Dr Jo Halliday from University of Glasgow will lead the field research for this project, other team members will also be involved. The research is funded by the Department for International Development (DFID) and five research councils in the UK.

## WHAT IS THE PURPOSE OF THIS STUDY?

You are being invited to take part in a research study to find out if animals in this area are carrying diseases that can also cause illness in people. In Tanzania, data collected by other studies has shown that brucellosis occurs in different types of animals (e.g., cattle, sheep and goats) and that brucellosis

is an important cause of disease in people. Brucellosis can be caused by several different types of *Brucella* bacteria. Each of these types of *Brucella* has different patterns of disease in animals. Information about which *Brucella* types are present in which animals in Tanzania is not well known. To develop plans for brucellosis control that are most likely to be effective and affordable, it is important to understand which animals are infected by which *Brucella* types, and which transmission routes are most important in transmitting brucellosis from animals to people. This three-year study will help to find out which *Brucella* types are found in different animal populations and what are the most important ways people become infected. The information collected will help develop control strategies and will help improve *Brucella* identification in Tanzania.

### WHY HAVE I BEEN CHOSEN?

You have been invited to take part in this study because you or someone that you know has fever. Fever is one of the main signs of brucellosis. We would like to do some tests to find out if you do have or have ever had brucellosis. We expect that about 360 people will take part in this study.

### DO I HAVE TO TAKE PART?

It is up to you to decide whether you do or do not take part in this study. Participation is voluntary. If you agree to take part in this study, you will be asked to sign, or add your thumbprint to this consent form. You will get a copy to keep. If you decide to take part, you are free to stop at any time and you do not need to give a reason for stopping. If you decide to stop taking part in this study your contributions to the study would be removed from any study outputs produced after the date that you stop taking part.

### WHAT WILL HAPPEN TO ME IF I TAKE PART?

If you decide to take part in this study you will be asked to allow a member of the project team to take blood for brucellosis testing. Up to 50cc of blood will be collected. The samples we collect, and tests we do, depend on whether you have fever currently. We will use the blood sample you give us to do tests for brucellosis and some other diseases including malaria. We will also use the sample to do more tests for brucellosis and other diseases at a later date. We will give you the results from some of these tests when they are available, if they tell us about current illness. We will not give you the results of all the tests that are done with your samples. All of these tests are free. You will also be asked to respond to some questions about you, your household and your health. It will take a few hours for us to collect samples and ask you some questions, and we will complete this either today or possibly in the next few days if there is not time to complete our questionnaire survey today. We would also like to return to your household in 4-6 weeks time so that we can collect a final blood sample for testing. You can choose to stop taking part at any time without any cost.

### WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART IN THE STUDY?

The collection of blood samples may cause some pain, bleeding or bruising where the needle enters the body. A small blood clot may form where the needle enters the body or there may be swelling in

the area. In a small number of people lightheadedness and fainting can also happen when a blood sample is collected.

**WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART IN THIS STUDY?**
The study team will also provide you with information about brucellosis. The results of the whole project will help to improve medical care and identification of diseases in Tanzania. We hope that the information collected through this study will be used to control brucellosis and reduce the impact that this disease has on human and animal health in Tanzania.

**WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?**
The samples we collect will firstly be tested at Kilimanjaro Clinical Research Institute in Moshi. After this, more tests may be done at one of our partner institutions including the Animal and Plant Health Agency of the UK and University of Glasgow. We will store leftover samples after brucellosis testing so that they can be used for studies in the future. This may include studies of other diseases that cause fever. We do not plan to contact you with the results from tests done on stored samples. This is because the studies will most likely be performed many months or years after the samples were collected and the results would no longer be important for your treatment. The results from this study may be used by local and international institutions, responsible ministries, individuals and scientists.

**WILL MY INFORMATION BE KEPT CONFIDENTIAL?**
All information which is collected about you, or responses that you provide during the course of the research study will be kept strictly confidential. You will not be identified by name in the study records shared outside KCMC or the University of Glasgow. If study results are given to other researchers, you will be identified by a code number, and any information about you will have your name and address removed so that you cannot be recognised from it.

**WHAT ABOUT COMPENSATION?**
No compensation will be provided for your participation in this study.

**WHAT ARE MY RIGHTS AS A RESEARCH SUBJECT?**
Taking part in this study is completely voluntary. You may choose not to take part in this study. Your decision will not affect your ability to take part in other studies or result in the loss of any benefits that you are entitled to.

**WHO HAS REVIEWED THIS STUDY?**
This study has been approved by the Ethics Committees of KCMC, the Tanzanian National Institute of Medical Research (NIMR) and the University of Glasgow.

**WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?**

For questions about this study contact: Dr Gabriel Shirima at 0763 973 003 or Dr Venance Maro at 0754 581 444. For questions about this study, to discuss problems, concerns or suggestions related to the study or to ask for information about this study, contact the Kilimanjaro Christian Medical Centre Ethics Committee at 027 275 3909. You can also contact the National Health Research Ethics Committee at 022 212 1400.

<u>**THANK YOU!**</u>
<u>**On behalf of the project team we would like to thank you for agreeing to take part in this study**</u>

**TUMAINI UNIVERSITY MAKUMIRA**

**KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE**

**P. O. Box 2240, Moshi   Tel. 027-27-53909**

Project Number:
Participant Unique Identification Number:

### CONSENT FORM

**Brucellosis research in northern Tanzania - Participant (Adults)**

"The purpose of this study, what will happen to me and the risks and benefits have been explained to me. **I** have been allowed to ask questions, and I am satisfied with the answers I have received. I have been told who to contact if I have questions, to discuss problems, or suggestions related to the study, or to receive more information about the study. I have read (or had read) this information sheet and agree to take part in this research study, with the understanding that I may withdraw at any time. I have been told that I will be given a signed and dated copy of this consent form."

**Please initial or mark box**

I confirm that I have read (or had read) and understand the information sheet for _____ participants, dated _____(version _____ ) for the above study and have had the opportunity to ask questions.

☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.

☐

I agree to take part in the above study.

☐

_____      _____      _____
**Name of participant (print)**              **Date**              **Signature**


_____      _____      _____
**Study staff conducting consent (print)**      **Date**              **Signature**


_____      _____      _____
**Witness' name (print) (As appropriate)**      **Date**              **Signature**

(1 copy for subject; 1 copy for researcher)

**University of Glasgow** | College of Medical, Veterinary & Life Sciences



## TUMAINI UNIVERSITY MAKUMIRA

## KILIMANJARO CHRISTIAN MEDICAL UNIVERSITY COLLEGE

### P. O. Box 2240, Moshi   Tel. 027-27-53909

Project Number:
Participant Unique Identification Number:

**CONSENT FORM**

**Brucellosis research in northern Tanzania – Participant (Minors)**

" The purpose of this study, what will happen to my child, and the risks and benefits have been explained to my child and me. I have been allowed to ask questions, and I am satisfied with the answers I have received. I have been told who to contact if I have questions, to discuss problems, or suggestions related to the study, or to receive more information about the study. I have read (or had read) this information sheet and agree for my child to take part in this research study, with the understanding that my child may withdraw at any time. I have been told that I will be given a signed and dated copy of this consent form."

**Please initial box**

I confirm that I have read (or had read) and understand the information sheet for
_____ participants, dated _____(version _____ ) for the above study
and have had the opportunity to ask questions.

I understand that my child's participation is voluntary and that we are free to withdraw at any time, without giving any reason or our legal rights being affected.

I agree for my child to take part in the above study.

Participant's name (print):     _____

_____     _____     _____
**Child Assent (print) (As appropriate)**      **Date**      **Signature**

_____     _____     _____
**Parent or Legal Guardian name (print)**      **Date**      **Signature**

_____     _____     _____
**Study staff conducting consent (print)**      **Date**      **Signature**

_____     _____     _____
**Witness' name (print) (As appropriate)**      **Date**      **Signature**
(1 copy for subject; 1 copy for researcher)

**Appendix 4: Bovine brucellosis Bayesian latent class model sensitivity analysis diagnostic plots**

# Bovine model: Model with uniform prior set and conditional dependence
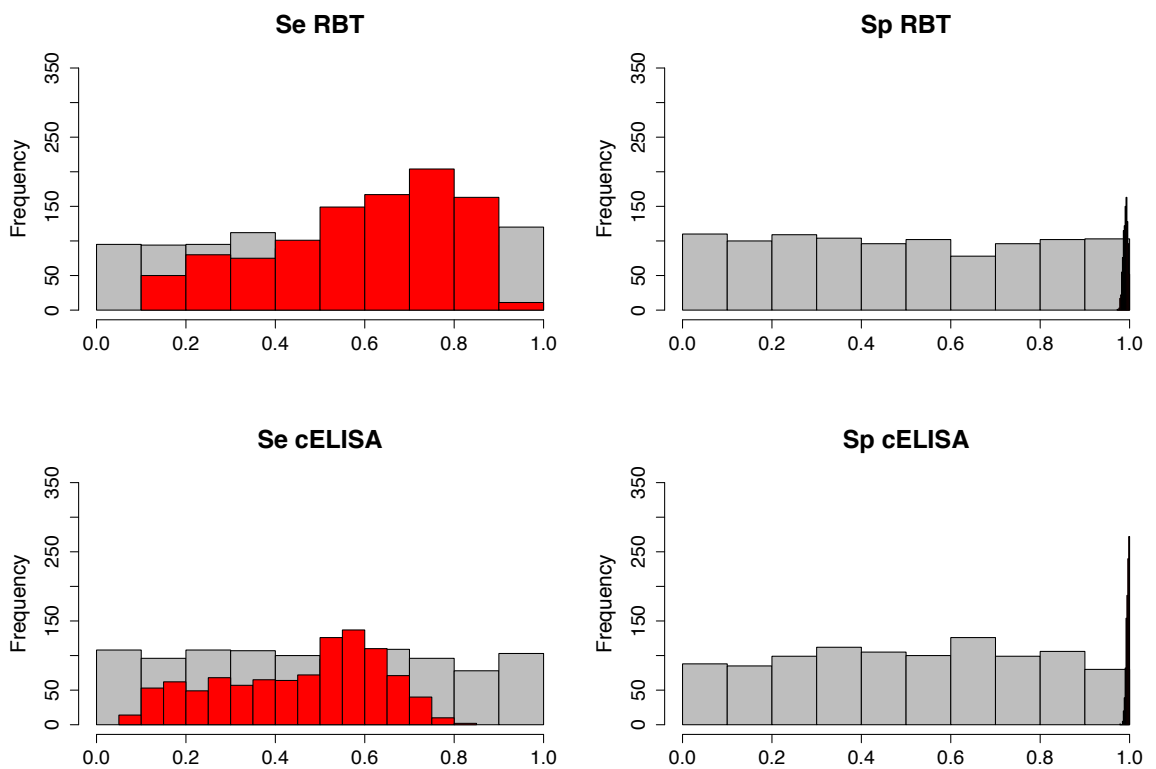


**Figure S4.1: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a bovine model under conditional dependence showing a uniform prior set\* (grey bars) and posterior estimates (red bars)**
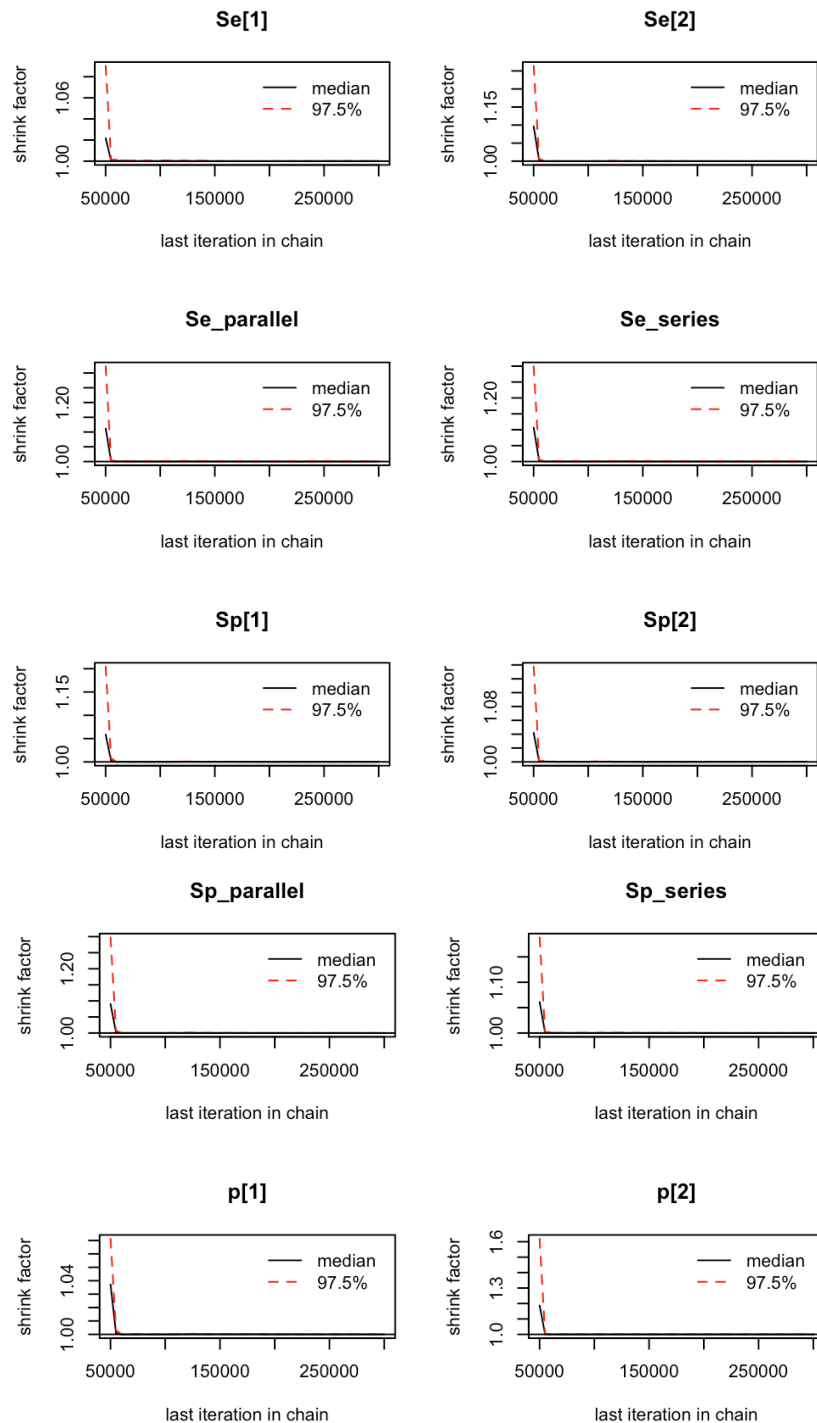
*uniform prior set:
Se RBT ~ dbeta(1,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(1,1)
Sp cELISA ~ dbeta(1,1)
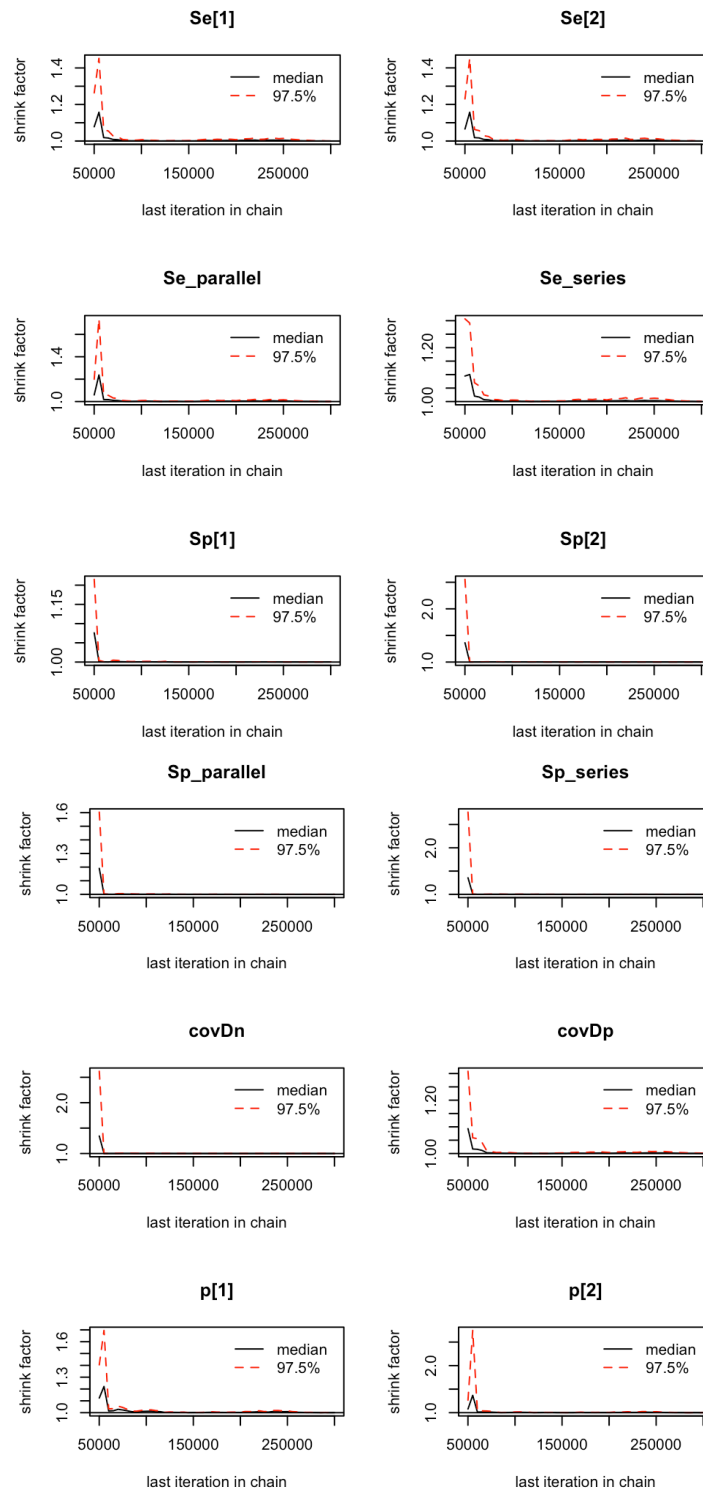
**Figure S4.2: Gelman-Rubin diagnostic plots showing convergence for each parameter of the bovine model with a uniform prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

**Bovine model: Model with uniform prior set and conditional independence**

**Figure S4.3: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a bovine model under conditional independence showing a uniform prior set\* (grey bars) and posterior estimates (red bars)**
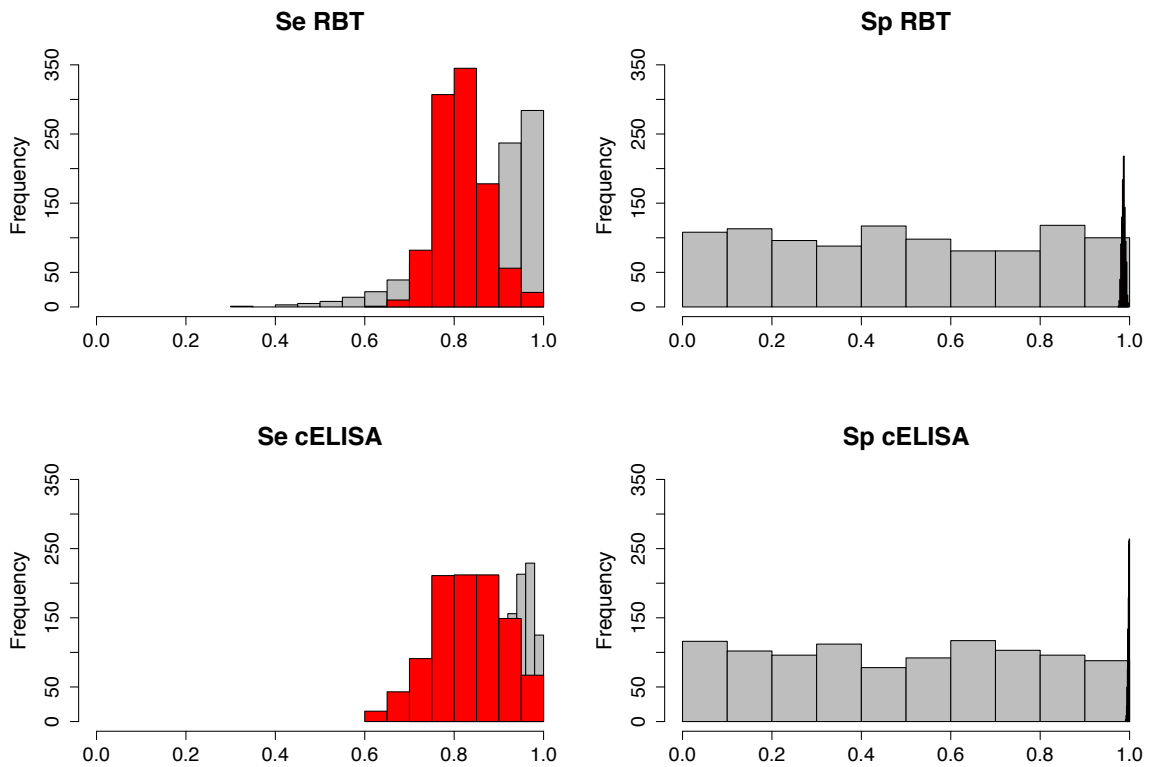\*uniform prior set:
Se RBT ~ dbeta(1,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(1,1)
Sp cELISA ~ dbeta(1,1)

**Figure S4.4: Gelman-Rubin diagnostic plots showing convergence for each parameter of the bovine model with a uniform prior set** and under conditional independence, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in aan exclusive pastoralist population*

# Bovine model: Model with relaxed prior set and conditional dependence



Figure S4.5: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a bovine model under conditional dependence showing a relaxed prior set* (grey bars) and posterior estimates (red bars)

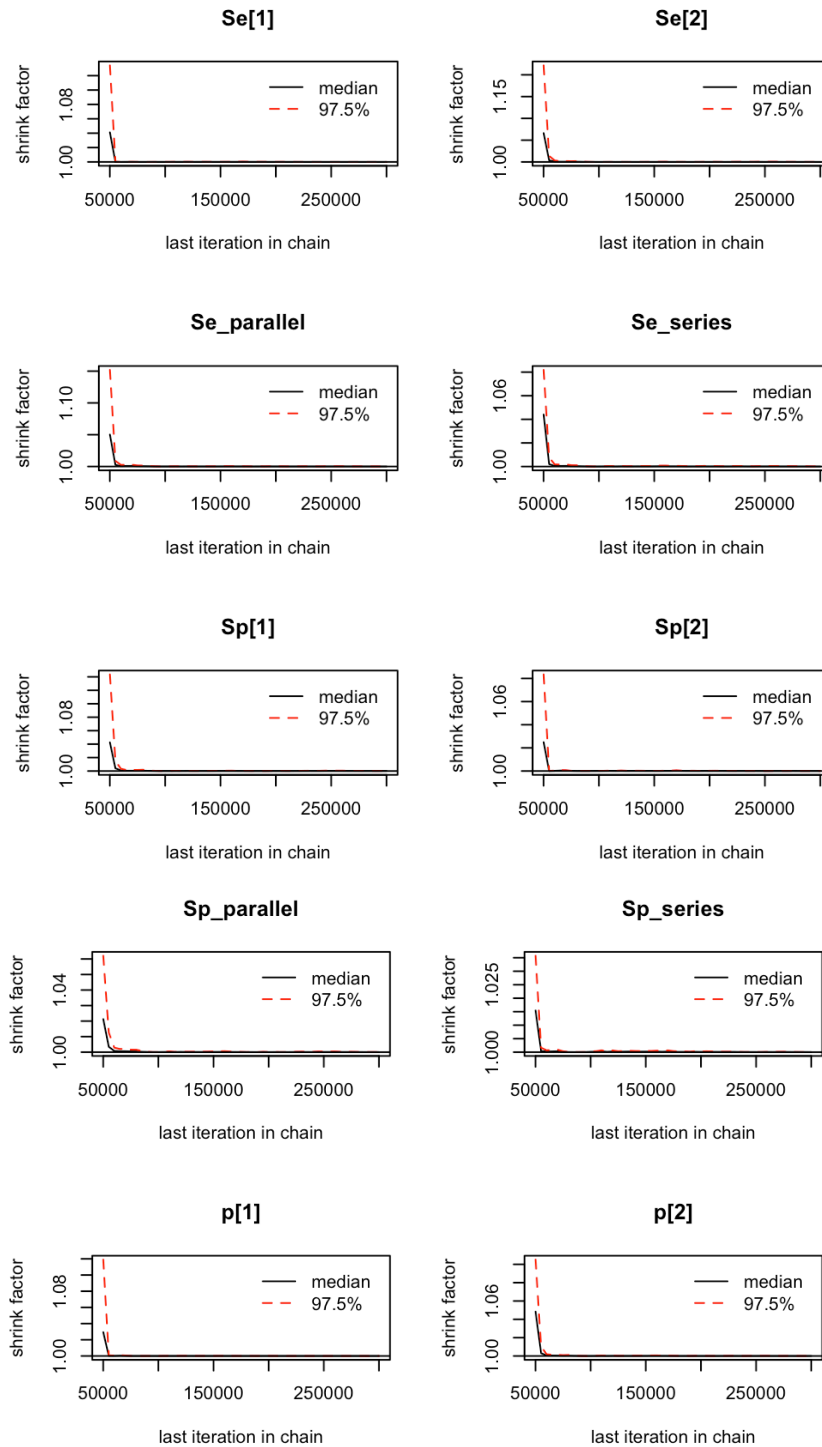*relaxed prior set:
Se RBT ~ dbeta(7,1)
Sp RBT ~ dbeta(1,1)
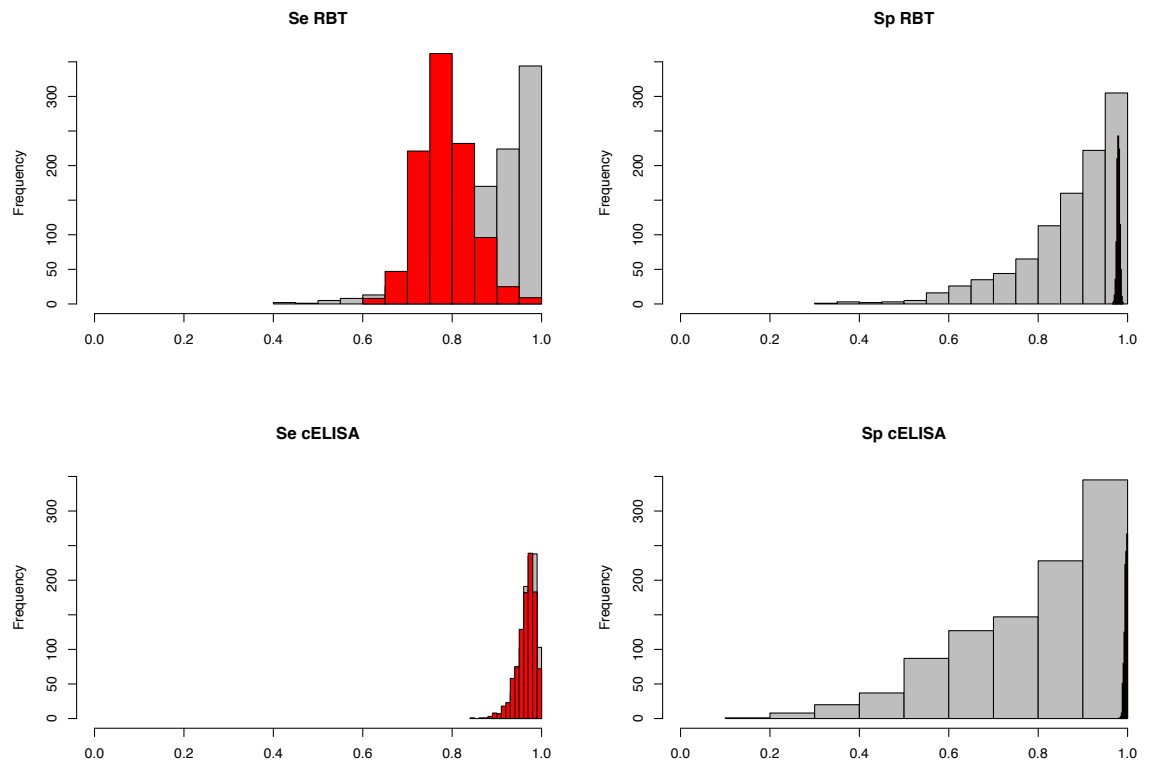Se cELISA ~ dbeta(30,2)
Sp cELISA ~ dbeta(1,1)
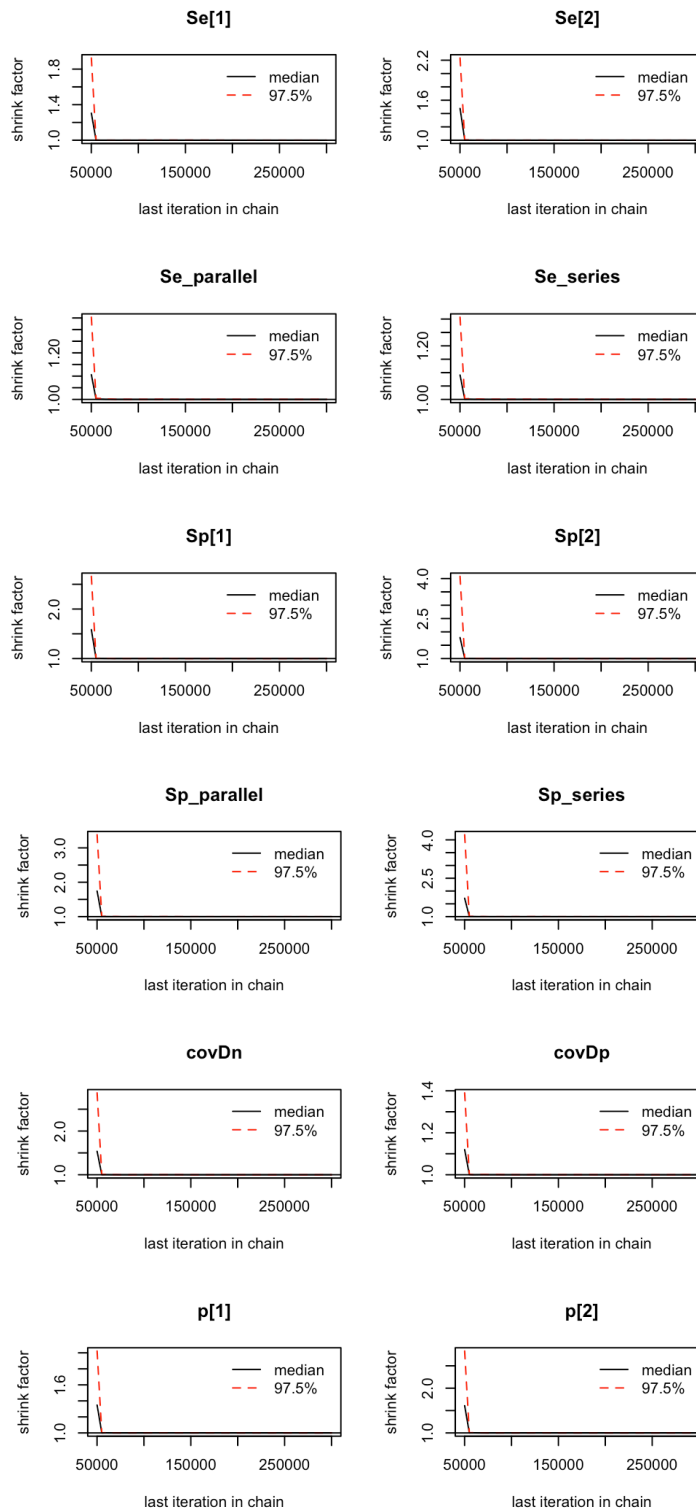
**Figure S4.6: Gelman-Rubin diagnostic plots showing convergence for each parameter of the bovine model with a relaxed prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

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# Bovine model: Model with relaxed prior set and conditional independence



Figure S4.7: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a bovine model under conditional independence showing a relaxed prior set* (grey bars) and posterior estimates (red bars)

*relaxed prior set:
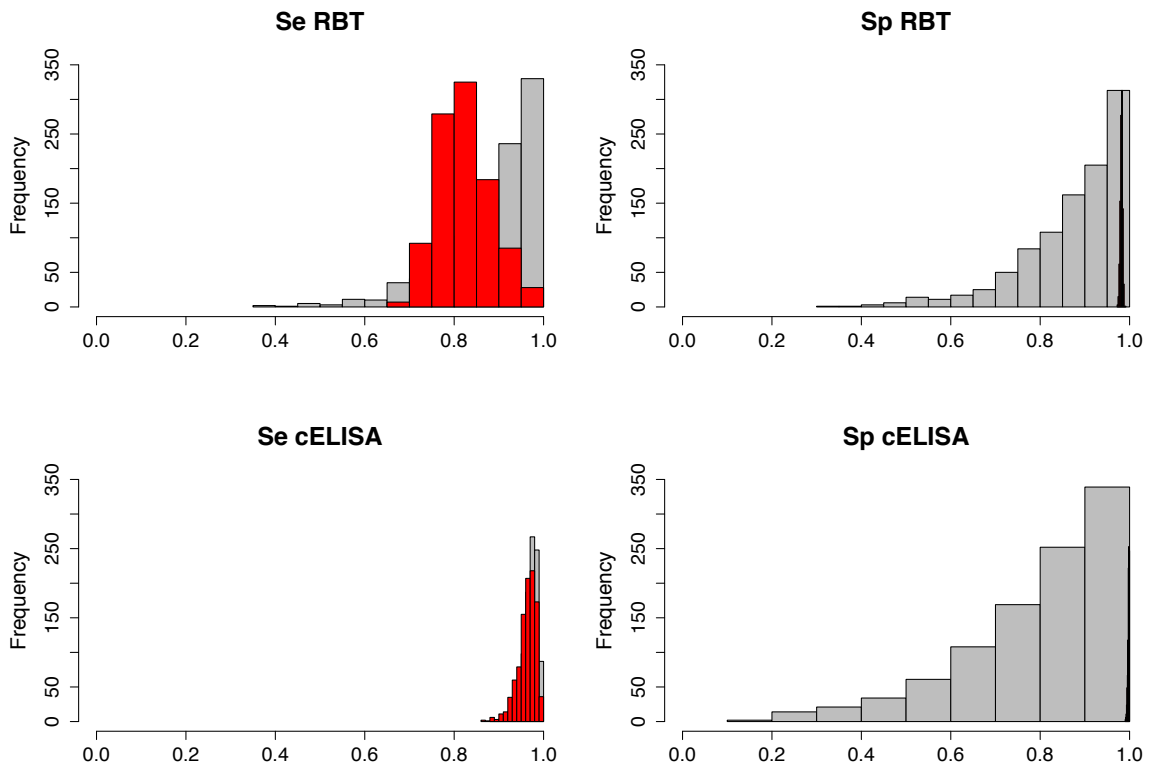Se RBT ~ dbeta(7,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(30,2)
Sp cELISA ~ dbeta(1,1)

**Figure S4.8: Gelman-Rubin diagnostic plots showing convergence for each parameter of the bovine model with a relaxed prior set and under conditional independence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Bovine model: Model with strict prior set and conditional dependence



**Figure S4.9: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a bovine model under conditional dependence showing a strict prior set\* (grey bars) and posterior estimates (red bars)**
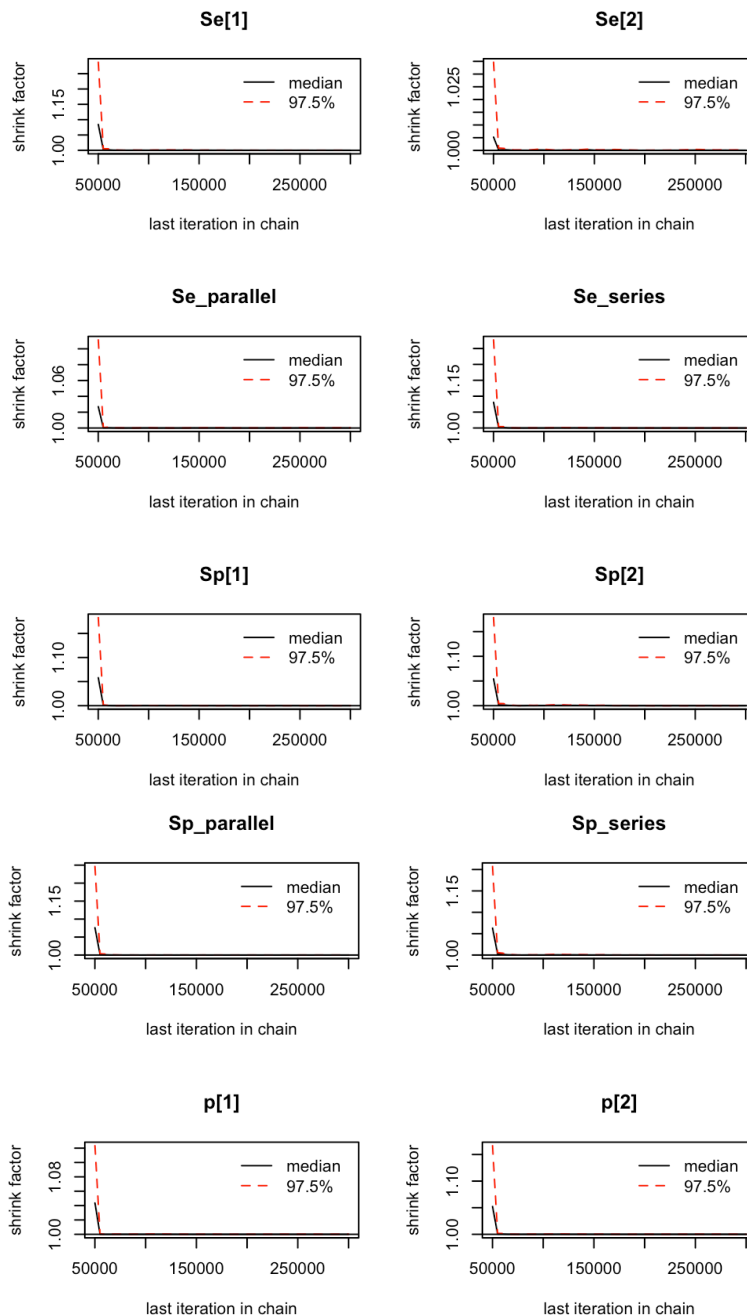\*strict prior set:
Se RBT ~ dbeta(8,1)
Sp RBT ~ dbeta(7,1)
Se cELISA ~ dbeta(102,3)
Sp cELISA ~ dbeta(4,1)
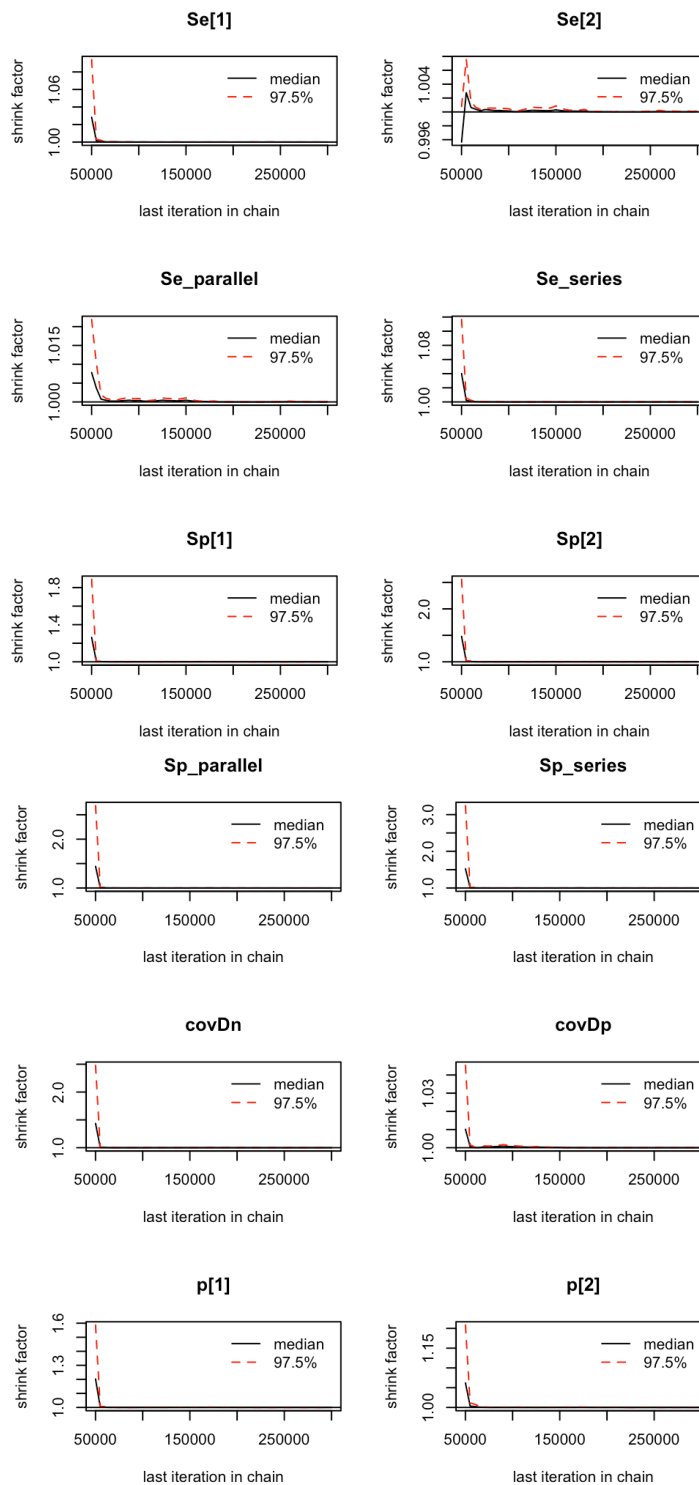
**Figure S4.10: Gelman-Rubin diagnostic plots showing convergence for each parameter of the bovine model with a strict prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Bovine model: Model with strict prior set and conditional independence



Figure S4.11: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a bovine model under conditional independence showing a strict prior set* (grey bars) and posterior estimates (red bars)
*strict prior set:
Se RBT ~ dbeta(8,1)
Sp RBT ~ dbeta(7,1)
Se cELISA ~ dbeta(102,3)
Sp cELISA ~ dbeta(4,1)

**Figure S4.12: Gelman-Rubin diagnostic plots showing convergence for each parameter of the bovine model with a strict prior set and under conditional independence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Appendix 5: Bayesian latent class model code: Bovine final model with conditional independence

**# Set up data for JAGS**
```
pop <- t(matrix(with(data, table(rbt, celisa, population),
            dnn=c("rbt", "celisa", "population")), 4,2))
n.pop = 2
n = apply(pop,1,sum)
```

**# JAGS model**
```
# [1] is RBT
# [2] is cELISA
# Tests in order  -- / +- / -+ / ++

cat("model{
    for (i in 1:n.pop){
    pop[i, 1:4] ~ dmulti(par[i, 1:4], n[i])
    p[i] ~ dunif(0, 0.49)
    par[i,4] <- p[i]*   Se[2] * Se[1] + (1-p[i])*(1-Sp[2])*(1-Sp[1]) #11
    par[i,3] <- p[i]*(1-Se[2])* Se[1] + (1-p[i])*  (Sp[2])*(1-Sp[1]) #01
    par[i,2] <- p[i]*   Se[2] * (1-Se[1]) + (1-p[i])*(1-Sp[2])*(Sp[1]) #10
    par[i,1] <- p[i]*(1-Se[2])* (1-Se[1]) + (1-p[i])*  (Sp[2])*(Sp[1]) #00
    }

    Se[1] ~ dbeta(7,1)
    Sp[1] ~ dbeta(1,1)
    Se[2] ~ dbeta(30,2)
    Sp[2] ~ dbeta(1,1)

    # To get in-series and in-parallel Se and Sp
    Se_series <- Se[1] * Se[2]
    Se_parallel <- 1 - (1 - Se[1]) * (1 - Se[2])
    Sp_series <- 1 - (1 - Sp[1]) * (1 - Sp[2])
    Sp_parallel <- Sp[1] * Sp[2]

    }", file="mod.jag")
```

**# Initial values for the three chains**
```
modelInit1 <- list(Se=c(0.4,0.99),  Sp=c(0.7,0.95),  p=c(0,0.1))
modelInit2 <- list(Se=c(0.3,0.98),  Sp=c(0.5,0.9),  p=c(0.01,0.25))
modelInit3 <- list(Se=c(0.2,0.8),  Sp=c(0.3,0.99),  p=c(0.02,0.49))
INI <- list(modelInit1, modelInit2,modelInit3)
```

**# Compile model components**

```
M <- jags.model(data=list(pop=pop,n=n, n.pop=n.pop), inits=INI, n.chains=3,
n.adapt= 50000, file="mod.jag")
```

**# Run the model with 50,000 burn-in and a further 250,000 iterations and thinning every 100th iteration**
```
R <- coda.samples(M, c("Se", "Sp", "p", "Se_series", "Se_parallel", "Sp_series",
"Sp_parallel"), n.iter=250000, n.thin=100)
```

**# Check model deviance information criterion (DIC)**
```
dic.samples(M, n.iter=250000, n.thin=100, type="pD")
```

**# Check model convergence**
```
densityplot(R)
gelman.diag(R, multivariate = FALSE)
gelman.plot(R)
traceplot(R)
```

**Appendix 6: Ovine brucellosis Bayesian latent class model sensitivity analysis diagnostic plots**

**Ovine model: Model with uniform prior set and conditional dependence**
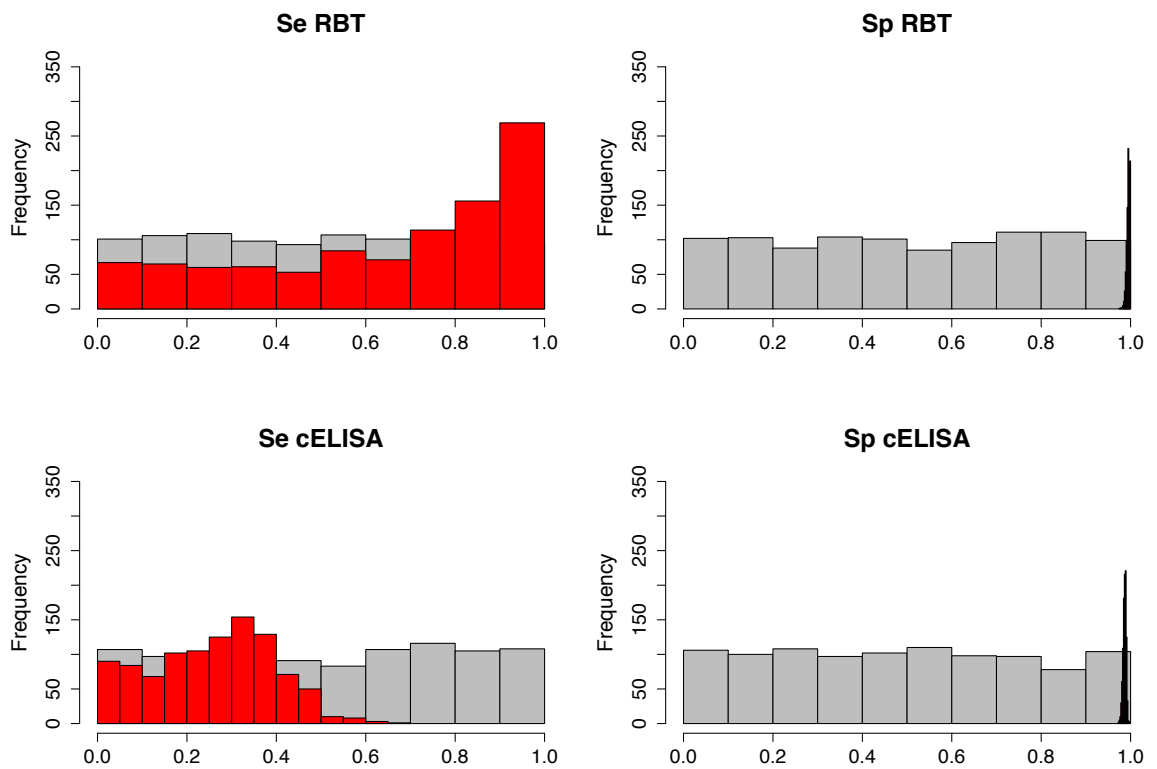
**Figure S6.1: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a ovine model under conditional dependence showing a uniform prior set\* (grey bars) and posterior estimates (red bars)**
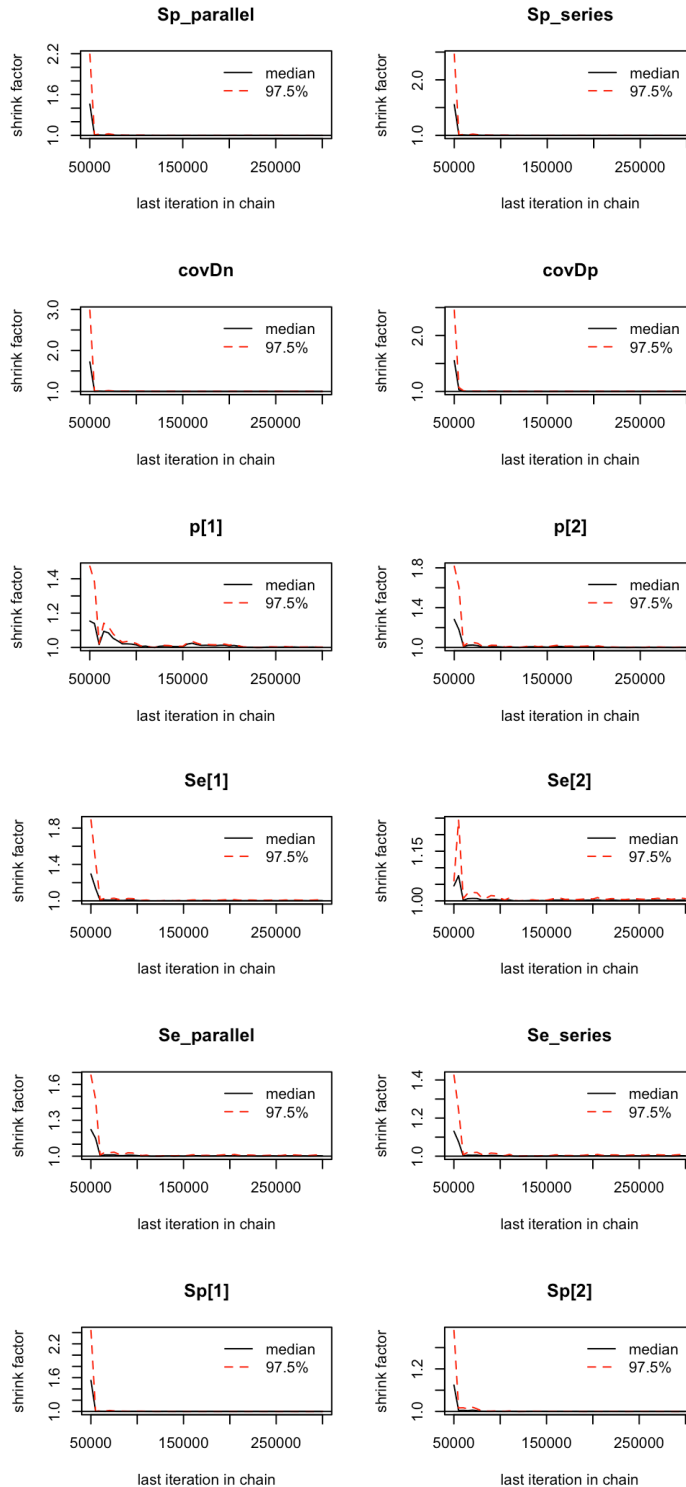\*uniform prior set:
Se RBT ~ dbeta(1,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(1,1)
Sp cELISA ~ dbeta(1,1)

**Figure S6.2: Gelman-Rubin diagnostic plots showing convergence for each parameter of the ovine model with a uniform prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Ovine model: Model with uniform prior set and conditional independence



**Figure S6.3: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a ovine model under conditional independence showing a uniform prior set\* (grey bars) and posterior estimates (red bars)**
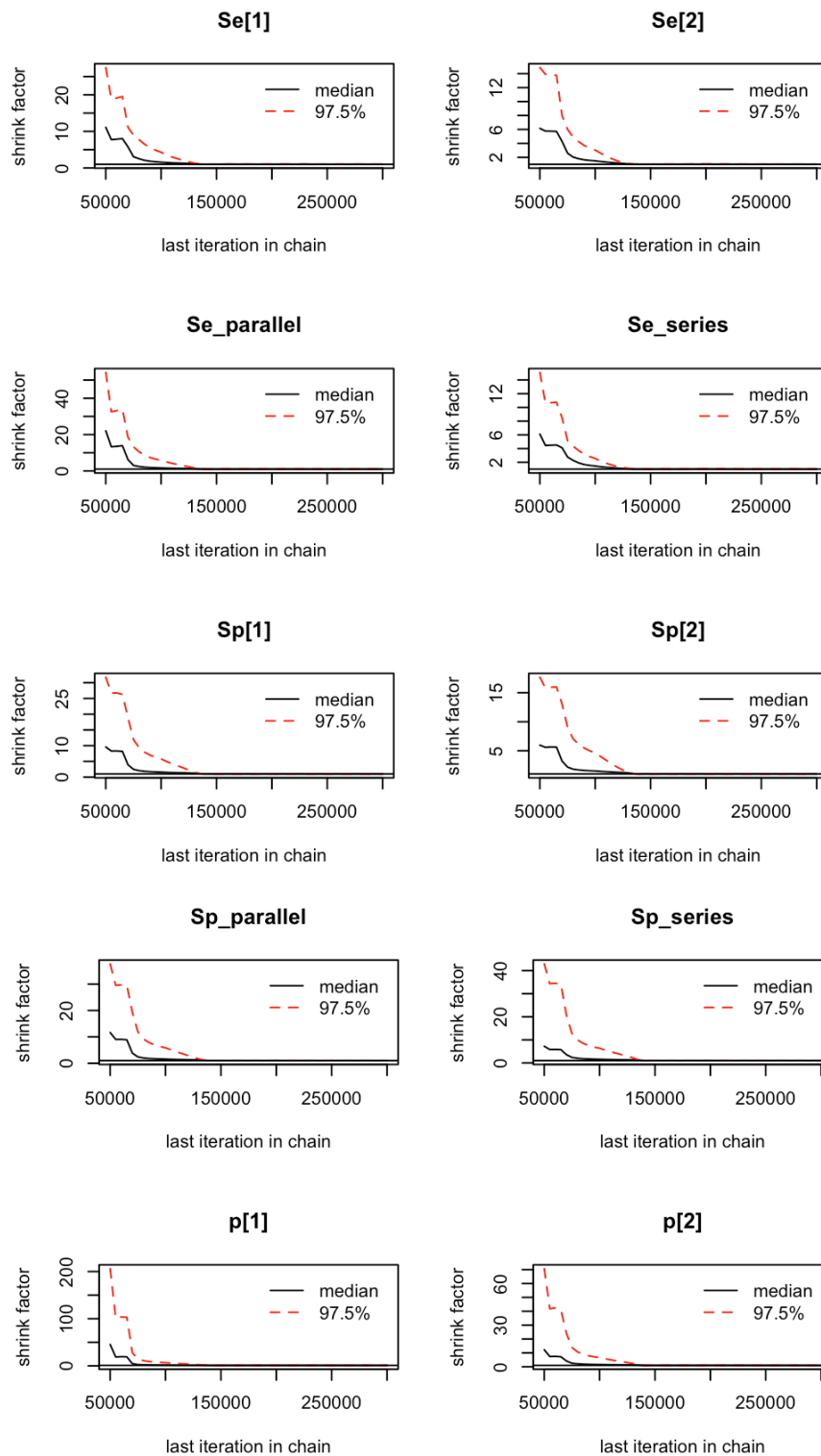
\*uniform prior set:
Se RBT ~ dbeta(1,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(1,1)
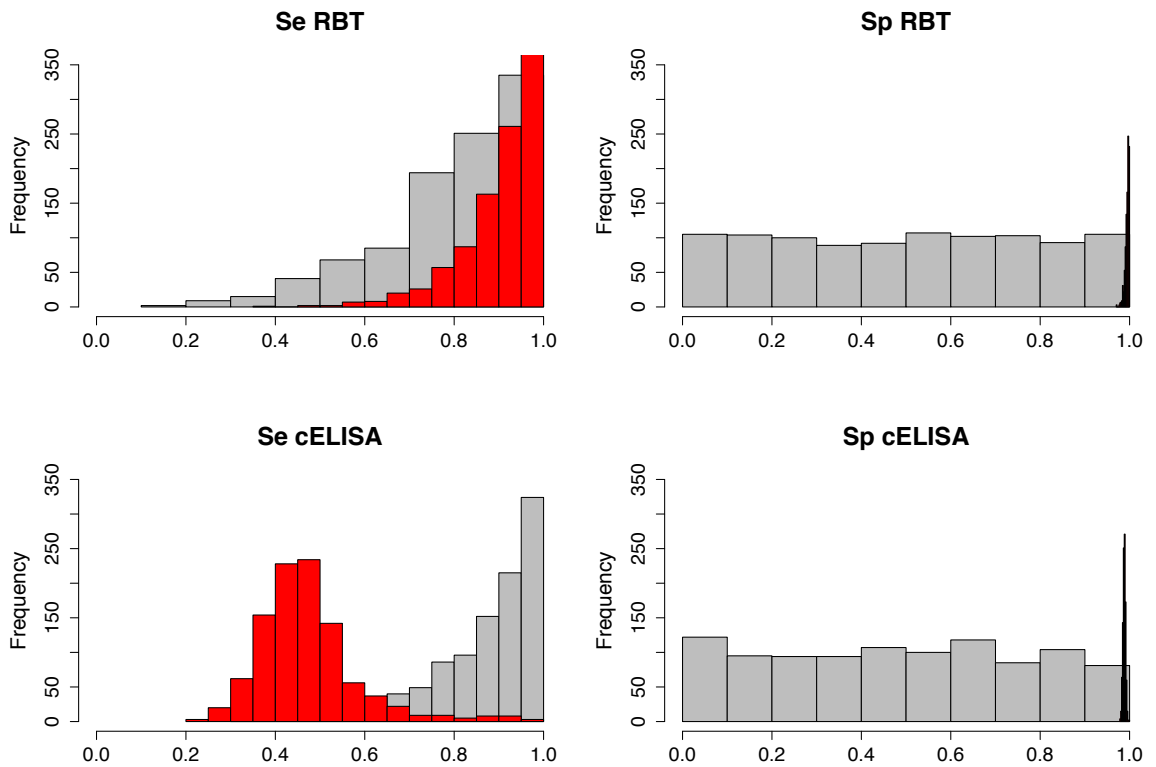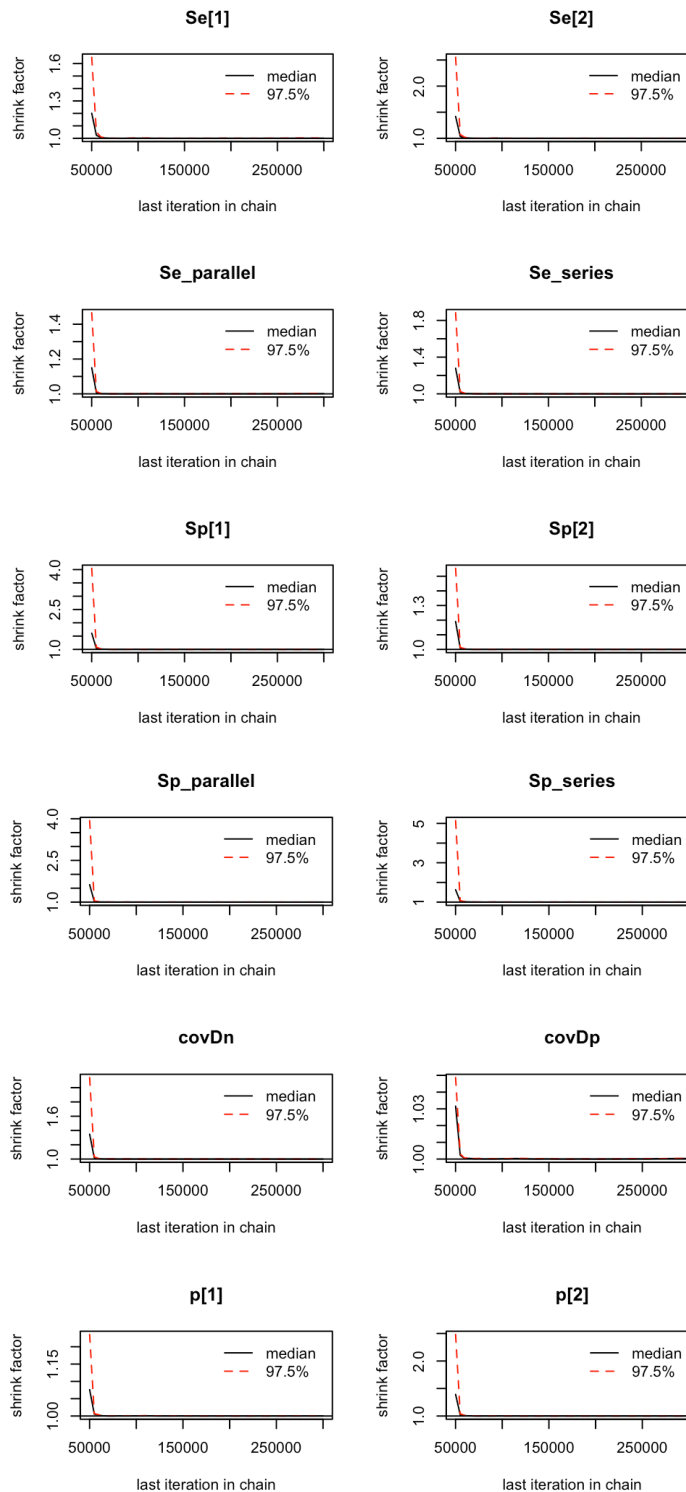Sp cELISA ~ dbeta(1,1)

**Figure S6.4: Gelman-Rubin diagnostic plots showing convergence for each parameter of the ovine model with a uniform prior set and under conditional independence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Ovine model: Model with relaxed prior set and conditional dependence
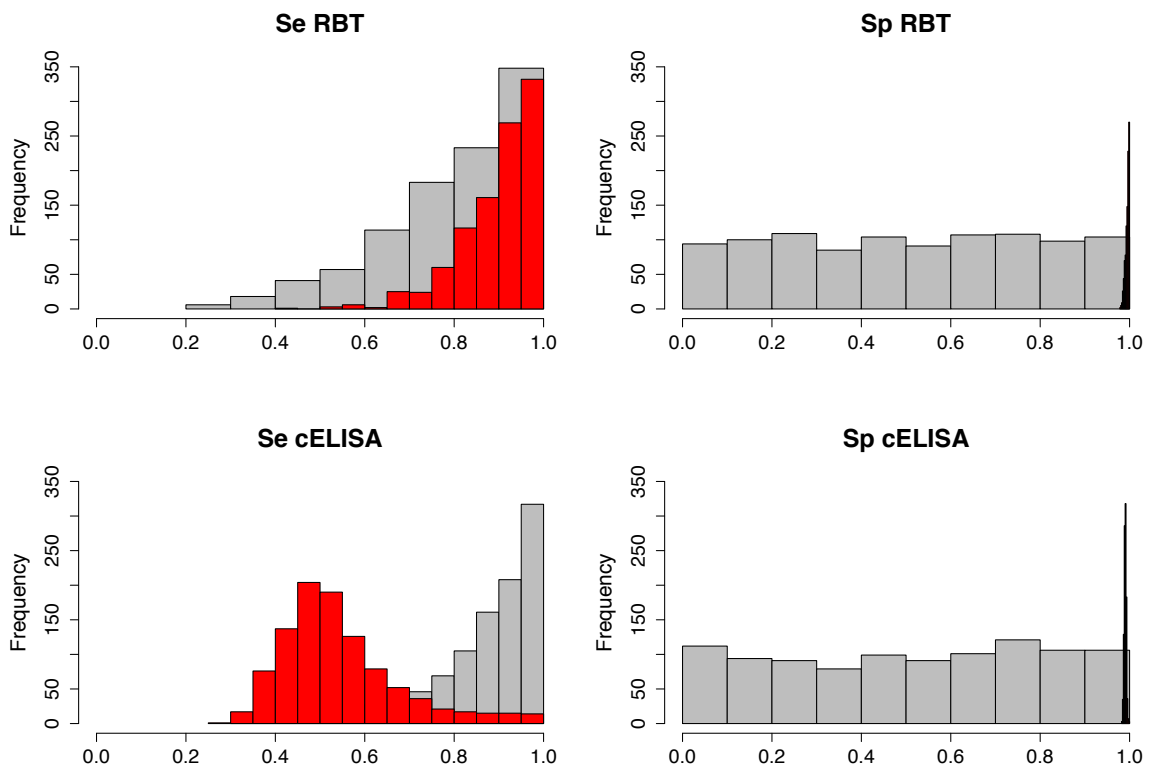


**Figure S6.5: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a ovine model under conditional dependence showing a relaxed prior set\* (grey bars) and posterior estimates (red bars)**
\*relaxed prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(1,1)

**Figure S6.6: Gelman-Rubin diagnostic plots showing convergence for each parameter of the ovine model with a relaxed prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Ovine model: Model with relaxed prior set and conditional independence



Figure S6.7: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a ovine model under conditional independence showing a relaxed prior set* (grey bars) and posterior estimates (red bars)
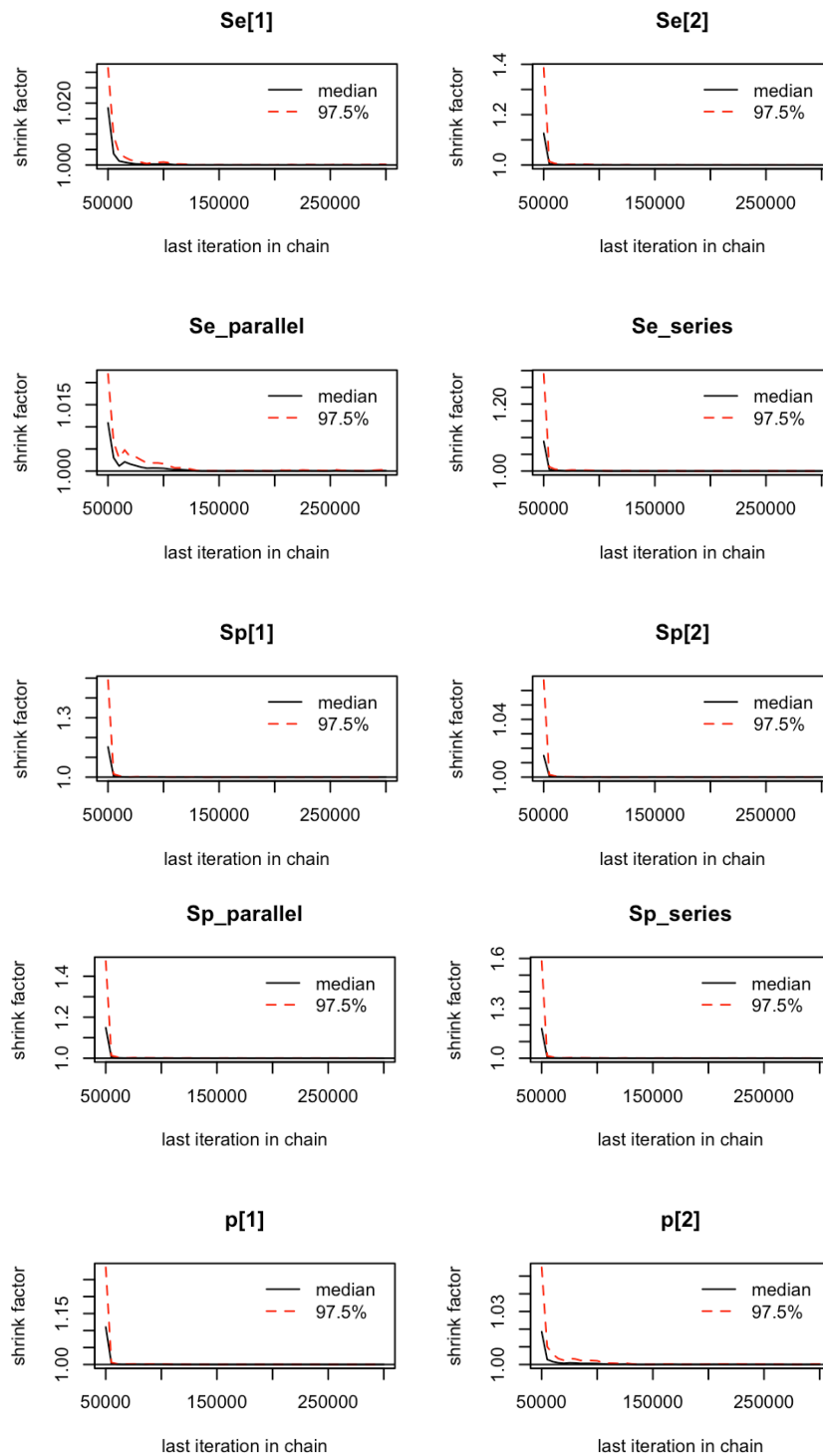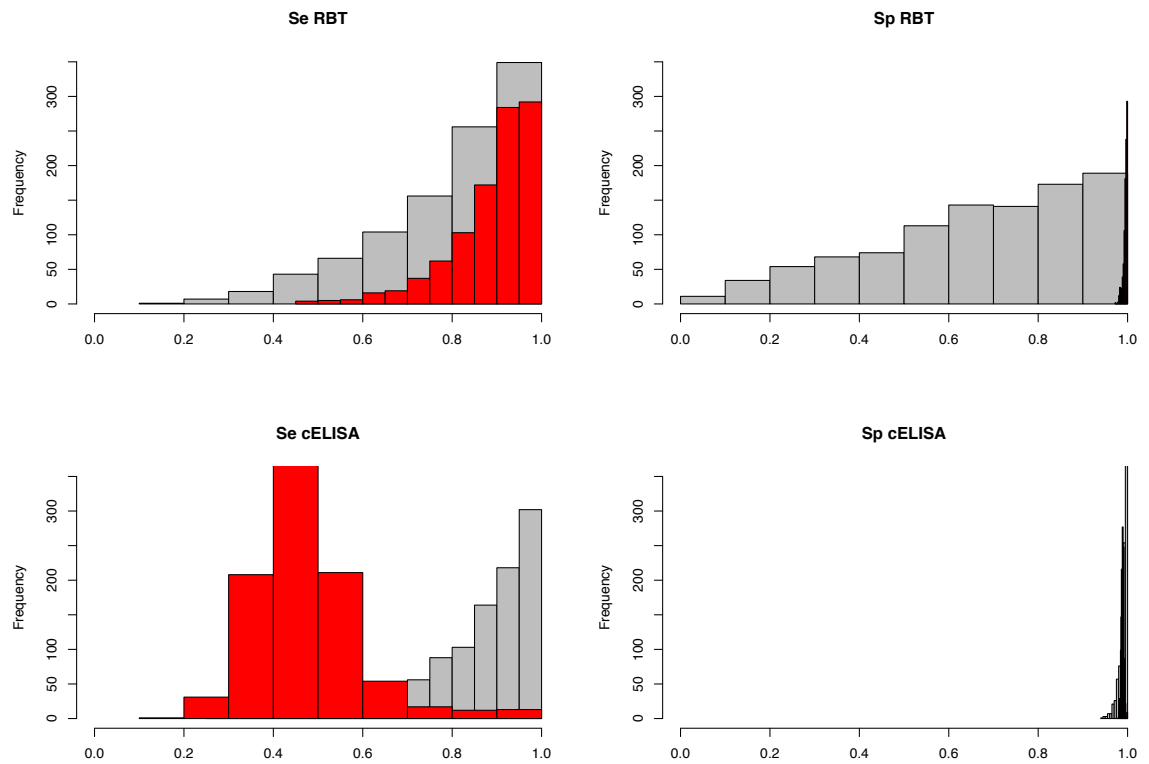
*relaxed prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(1,1)

**Figure S6.8: Gelman-Rubin diagnostic plots showing convergence for each parameter of the ovine model with a relaxed prior set and under conditional independence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

**Figure S6.9: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a ovine model under conditional dependence showing a strict prior set* (grey bars) and posterior estimates (red bars)**
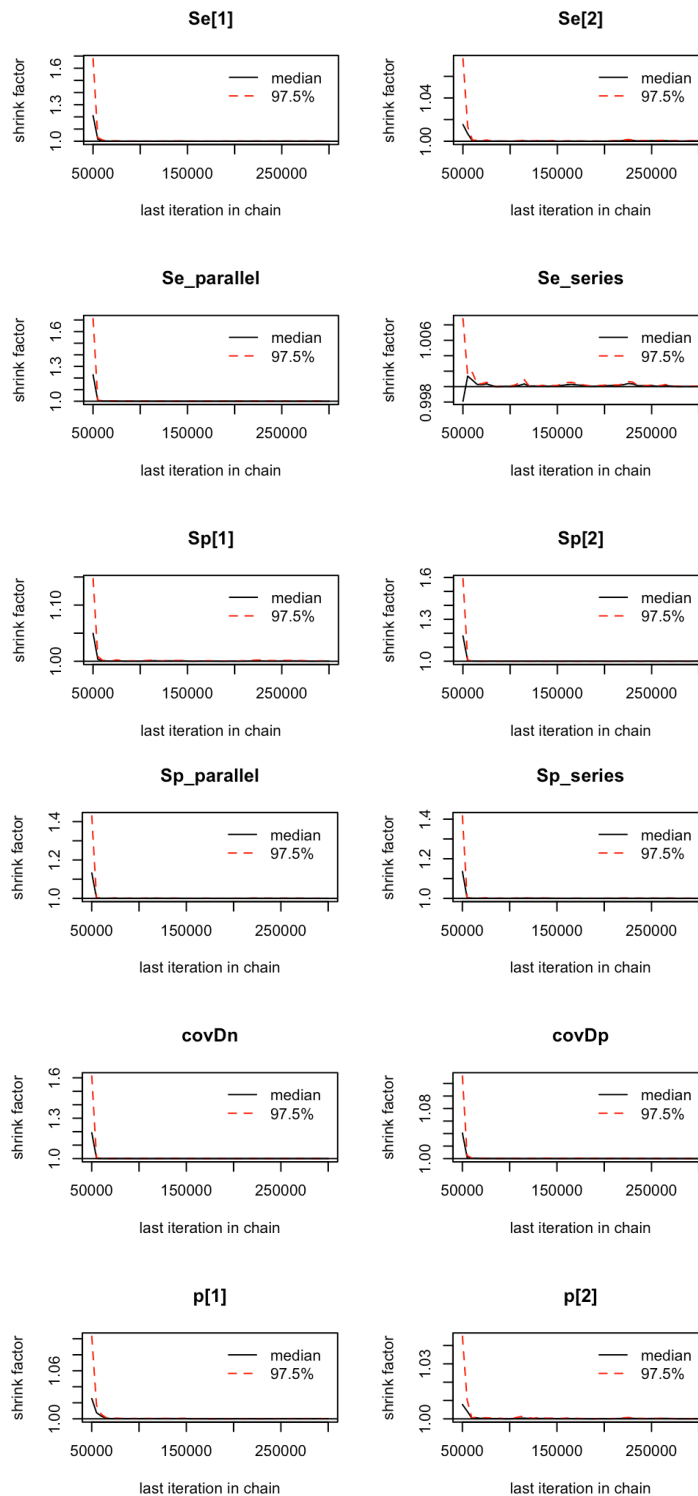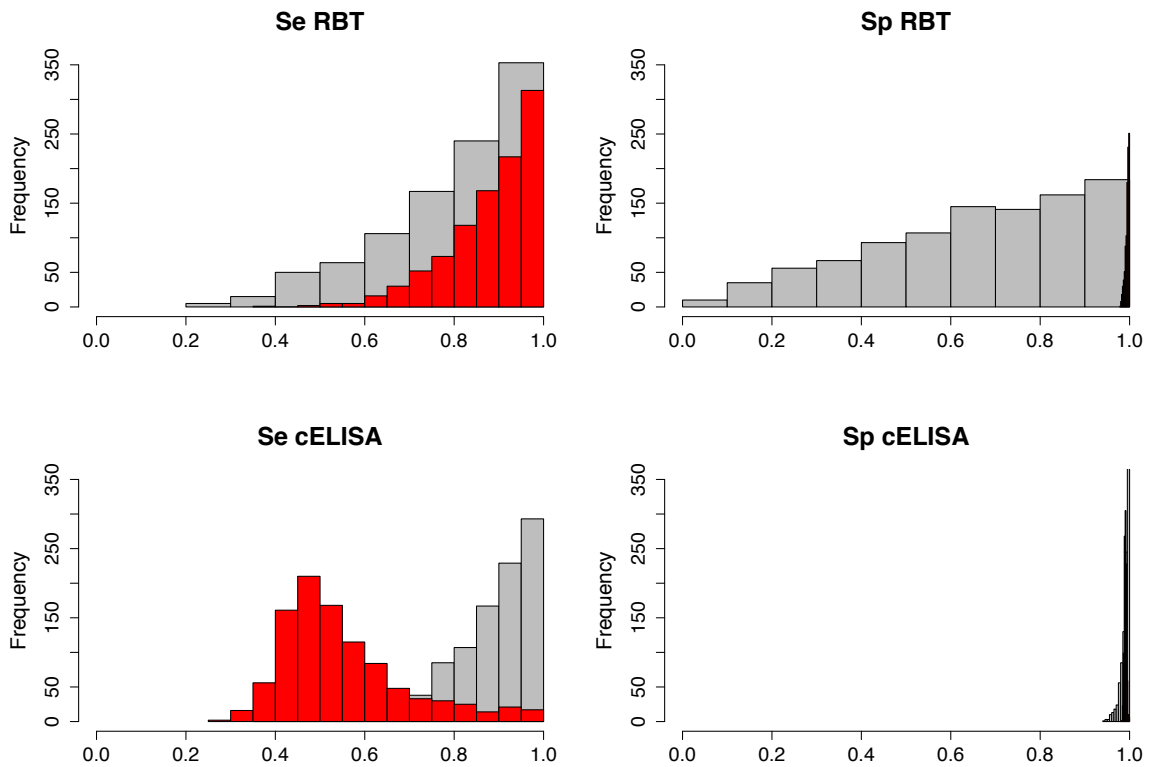*strict prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(2,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(104,1)

**Figure S6.10: Gelman-Rubin diagnostic plots showing convergence for each parameter of the ovine model with a strict prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Ovine model: Model with strict prior set and conditional independence



**Figure S6.11: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a ovine model under conditional independence showing a strict prior set\* (grey bars) and posterior estimates (red bars)**
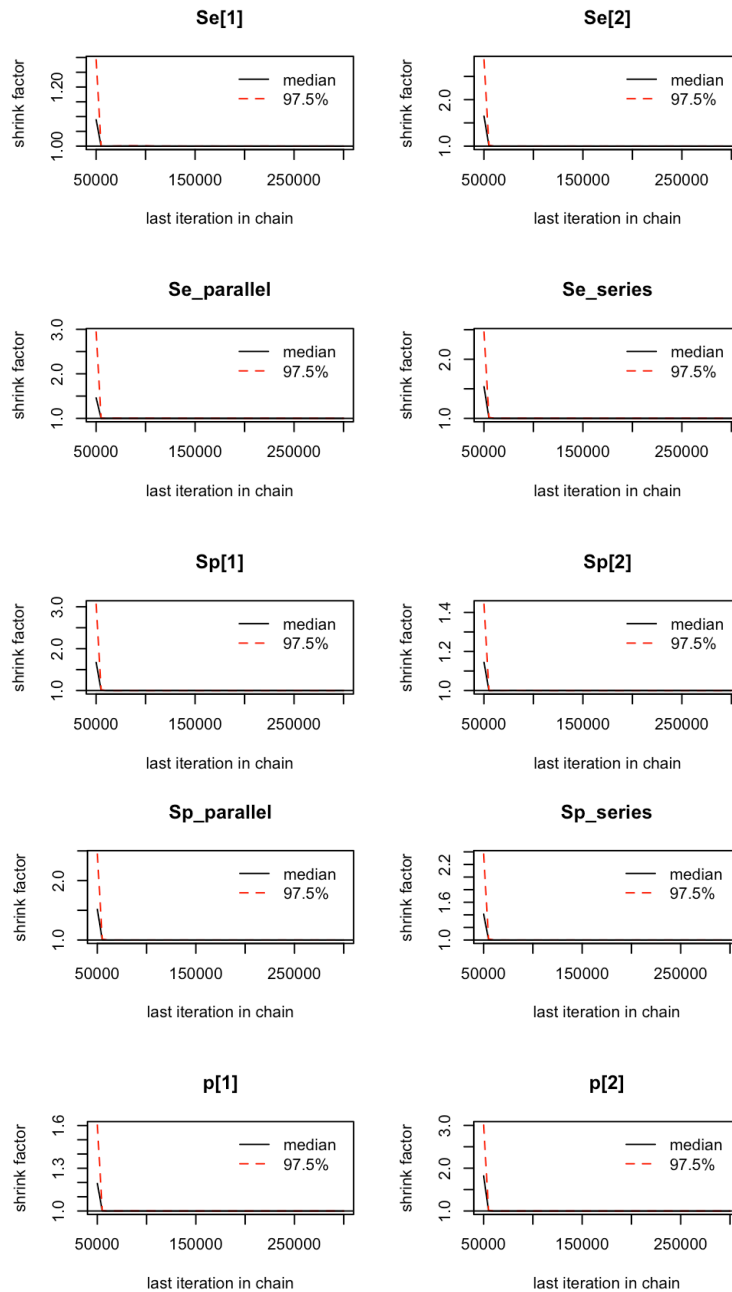\*strict prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(2,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(104,1)

**Figure S6.12: Gelman-Rubin diagnostic plots showing convergence for each parameter of the ovine model with a strict prior set and under conditional independence***, Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is* specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population

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**Appendix 7: Ovine final model diagnostic plots**
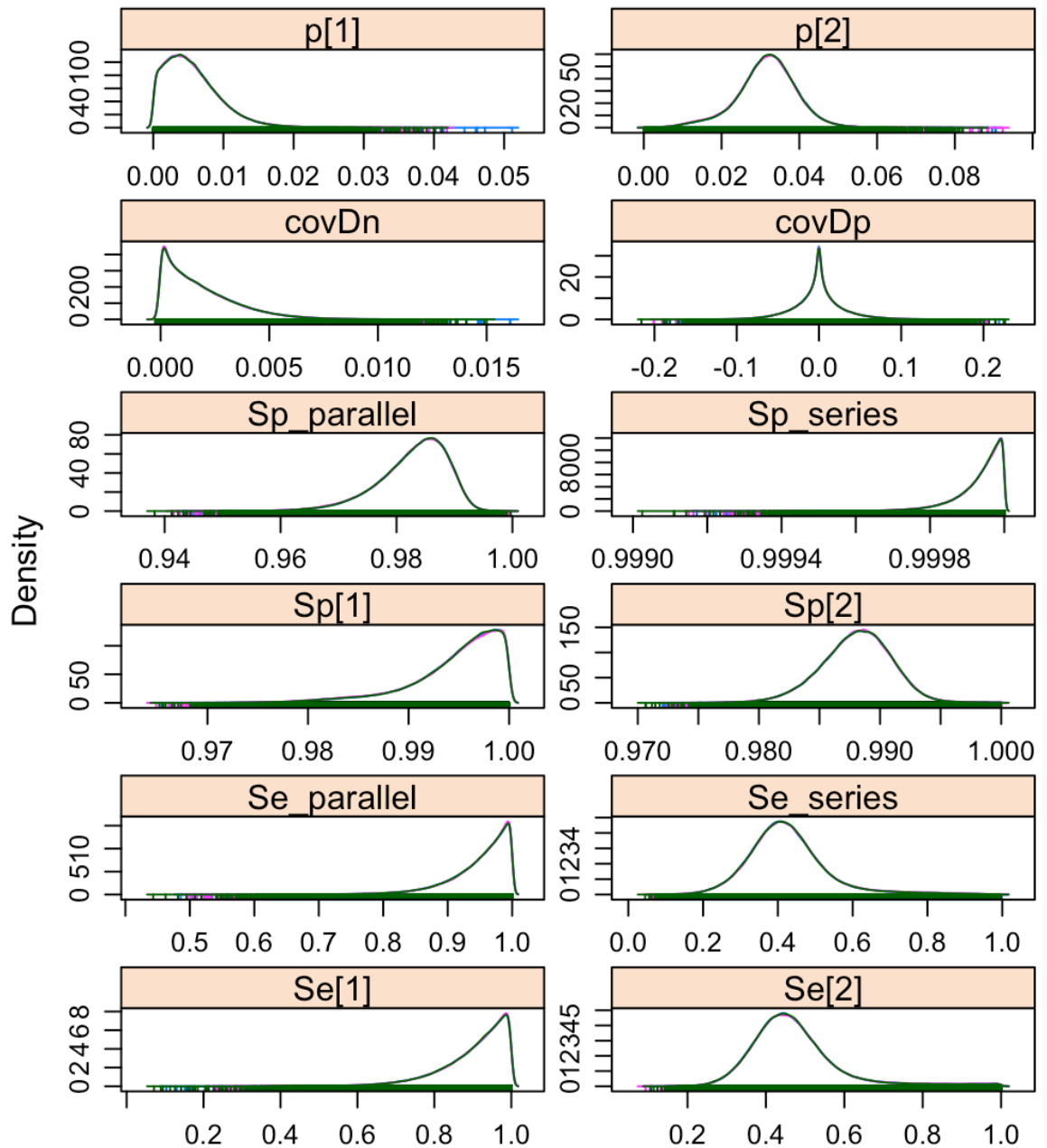
**Figure S7.1: Density plots for each parameter of the final ovine model,** *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

**Figure S7.2: Gelman-Rubin diagnostic plots showing convergence for each parameter of the final ovine model**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

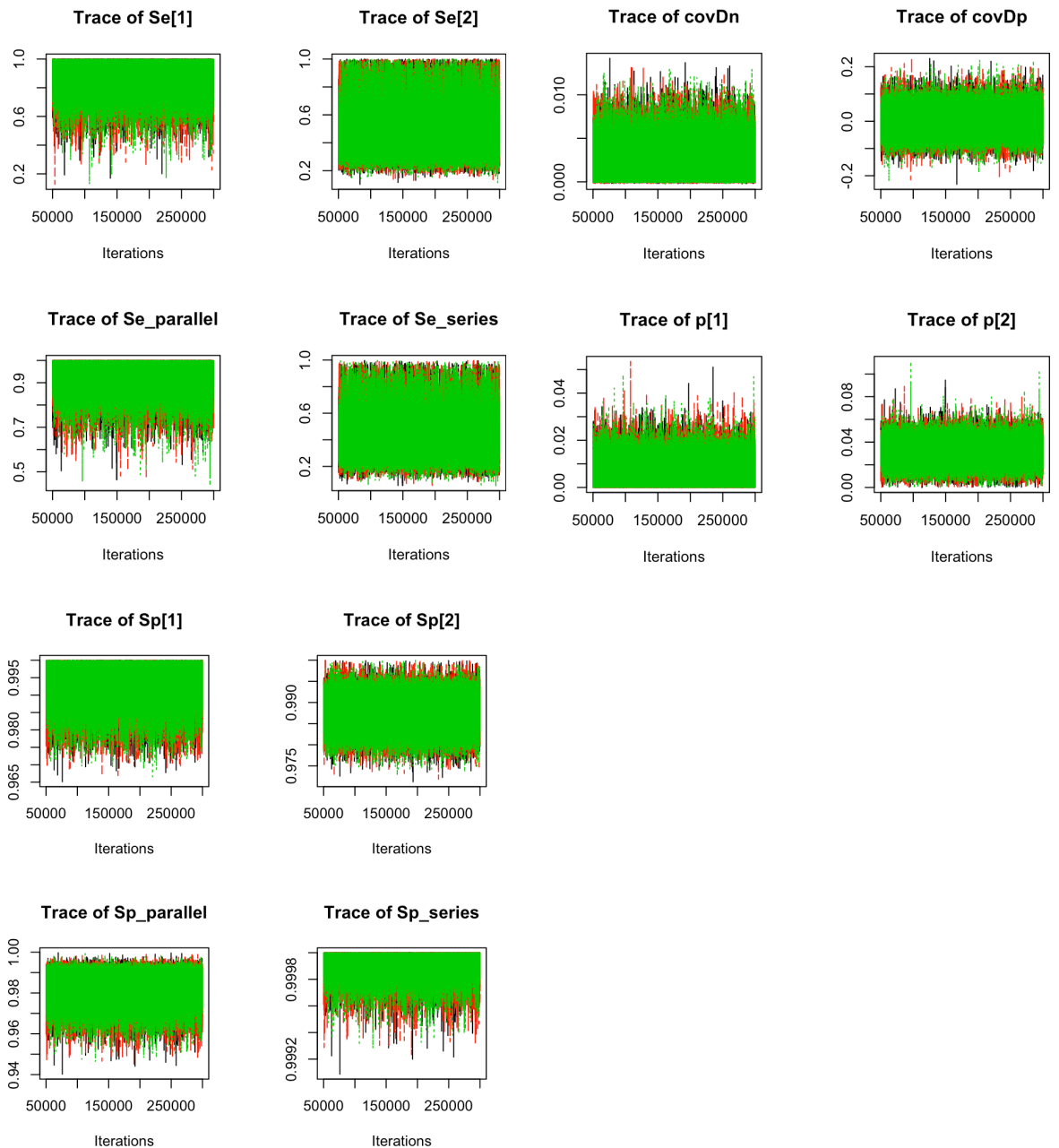**Figure S7.3: Trace plots showing MCMC chain convergence for each parameter of the final ovine model**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

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# Appendix 8: Bayesian latent class model code: Ovine final model with conditional dependence

**# Set up data for JAGS**

```
pop <- t(matrix(with(data, table(rbt, celisa, population),
               dnn=c("rbt", "celisa", "population")), 4,2))
n.pop = 2
n = apply(pop,1,sum)
```

**# JAGS model**
```
# [1] is RBT
# [2] is cELISA
# Tests in order  -- / +- / -+ / ++

cat("model{
    for (i in 1:n.pop){
    pop[i, 1:4] ~ dmulti(par[i, 1:4], n[i])
    p[i] ~ dunif(0, 0.49)
    par[i,4] <- p[i]*   (Se[2] * Se[1] + covDp) + (1-p[i])*((1-Sp[2])*(1-Sp[1]) + covDn)
    par[i,3] <- p[i]* ((1-Se[2])* Se[1] - covDp) + (1-p[i])*((Sp[2])*(1-Sp[1]) - covDn)
    par[i,2] <- p[i]*   (Se[2] * (1-Se[1]) - covDp) + (1-p[i])*((1-Sp[2])*(Sp[1]) -
covDn)
    par[i,1] <- p[i]* ((1-Se[2])* (1-Se[1]) + covDp) + (1-p[i])*((Sp[2])*(Sp[1]) +
covDn)
    }

    ls <- (Se[1]-1)*(1-Se[2])
    us <- min(Se[1],Se[2]) - Se[1]*Se[2]
    lc <- (Sp[1]-1)*(1-Sp[2])
    uc <- min(Sp[1],Sp[2]) - Sp[1]*Sp[2]
    rhoD <- covDp / sqrt(Se[1]*(1-Se[1])*Se[2]*(1-Se[2]))
    rhoDc <- covDn / sqrt(Sp[1]*(1-Sp[1])*Sp[2]*(1-Sp[2]))

    Se[1] ~ dbeta(4,1)
    Sp[1] ~ dbeta(1,1)
    Se[2] ~ dbeta(7,1)
    Sp[2] ~ dbeta(1,1)

    # To get Serial and Parallel Ses and Sps
    Se_series <- Se[1] * Se[2]
    Se_parallel <- 1 - (1 - Se[1]) * (1 - Se[2])
    Sp_series <- 1 - (1 - Sp[1]) * (1 - Sp[2])
    Sp_parallel <- Sp[1] * Sp[2]
    covDn ~ dunif(lc, uc)
```

```
    covDp ~ dunif(ls, us)

    }", file="mod.jag")
```

**# Initial values for the three chains**
```
modelInit1 <- list(Se=c(0.7,0.99),  Sp=c(0.3,0.99),  p=c(0,0.1))
modelInit2 <- list(Se=c(0.3,0.8), Sp=c(0.15,0.7),  p=c(0.01,0.25))
modelInit3 <- list(Se=c(0.6,0.95),  Sp=c(0.4,0.90),  p=c(0.02,0.49))
INI <- list(modelInit1, modelInit2,modelInit3)
```

**# Compile model components**
```
M <- jags.model(data=list(pop=pop,n=n, n.pop=n.pop), inits=INI, n.chains=3,
n.adapt= 50000, file="mod.jag")
```

**# Run the model with 50,000 burn-in and a further 250,000 iterations and thinning every 100$^{th}$ iteration**
```
R <- coda.samples(M, c("Se", "Sp", "p", "Se_series", "Se_parallel", "Sp_series",
"Sp_parallel"), n.iter=250000, n.thin=100)
```

**# Check model deviance information criterion (DIC)**
```
dic.samples(M, n.iter=250000, n.thin=100, type="pD")
```

**# Check model convergence**
```
densityplot(R)
gelman.diag(R, multivariate = FALSE)
gelman.plot(R)
traceplot(R)
```

# Appendix 9: Caprine brucellosis Bayesian latent class model sensitivity analysis diagnostic plots

# Caprine model: Model with uniform prior set and conditional dependence
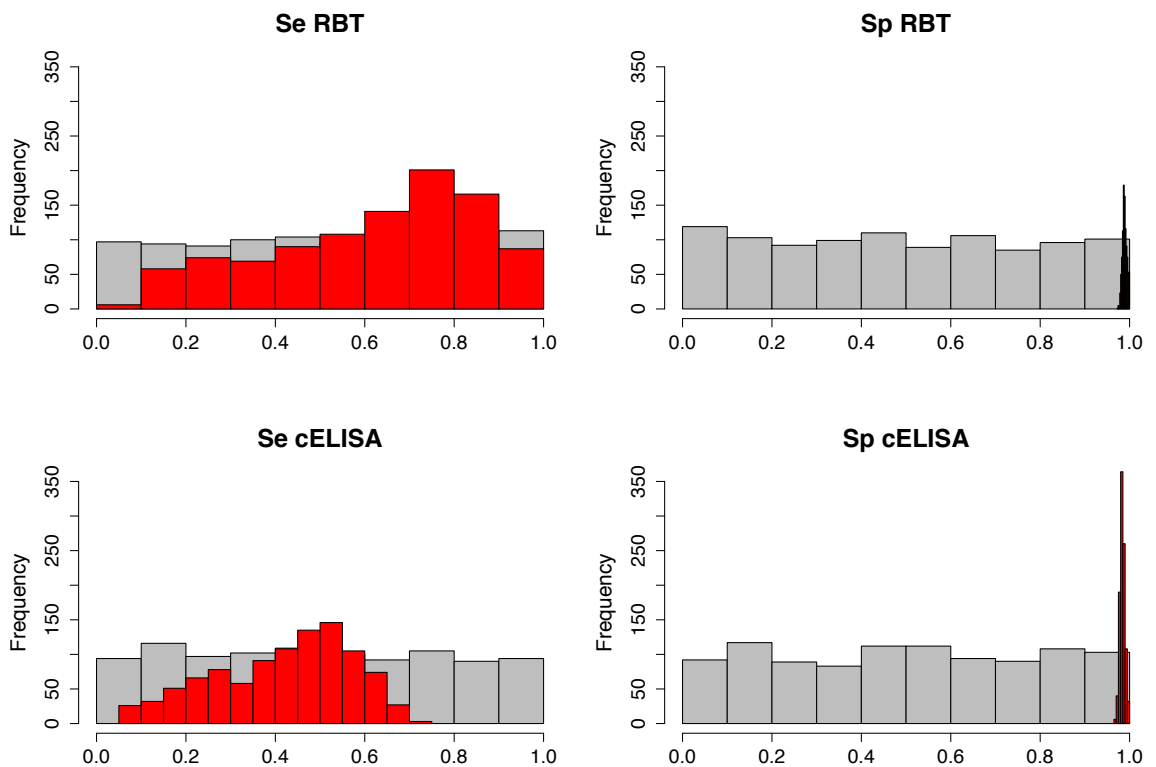


Figure S9.1: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a caprine model under conditional dependence showing a uniform prior set* (grey bars) and posterior estimates (red bars)

*uniform prior set:
Se RBT ~ dbeta(1,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(1,1)
Sp cELISA ~ dbeta(1,1)

**Figure S9.2: Gelman-Rubin diagnostic plots showing convergence for each parameter of the caprine model with a uniform prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Caprine model: Model with uniform prior set and conditional independence



**Figure S9.3: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a caprine model under conditional independence showing a uniform prior set\* (grey bars) and posterior estimates (red bars)**
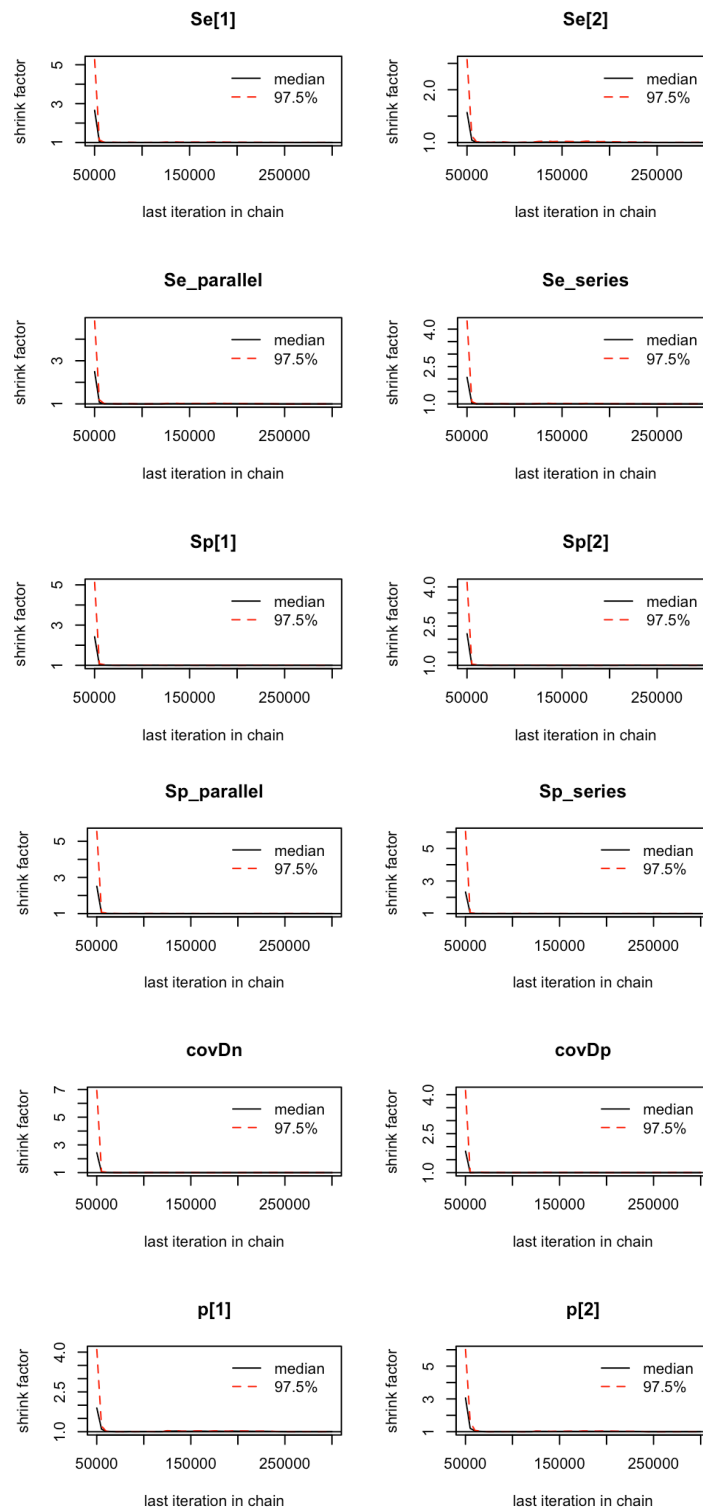\*uniform prior set:
Se RBT ~ dbeta(1,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(1,1)
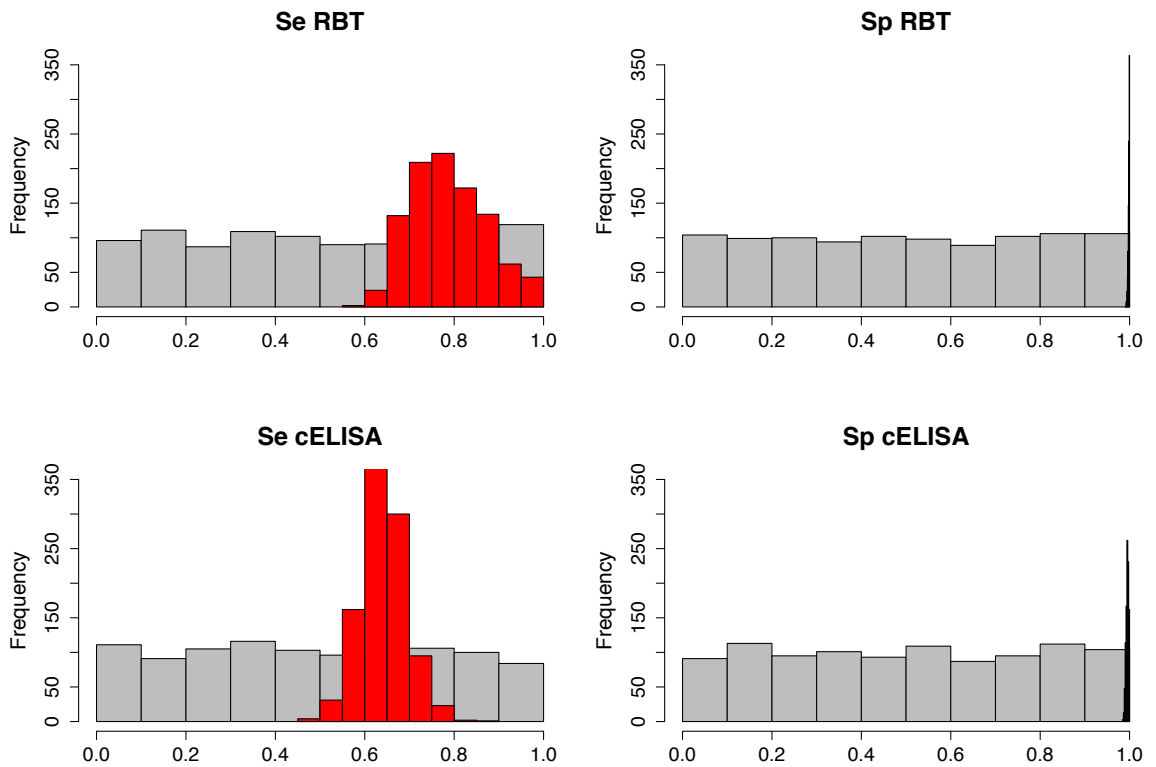Sp cELISA ~ dbeta(1,1)
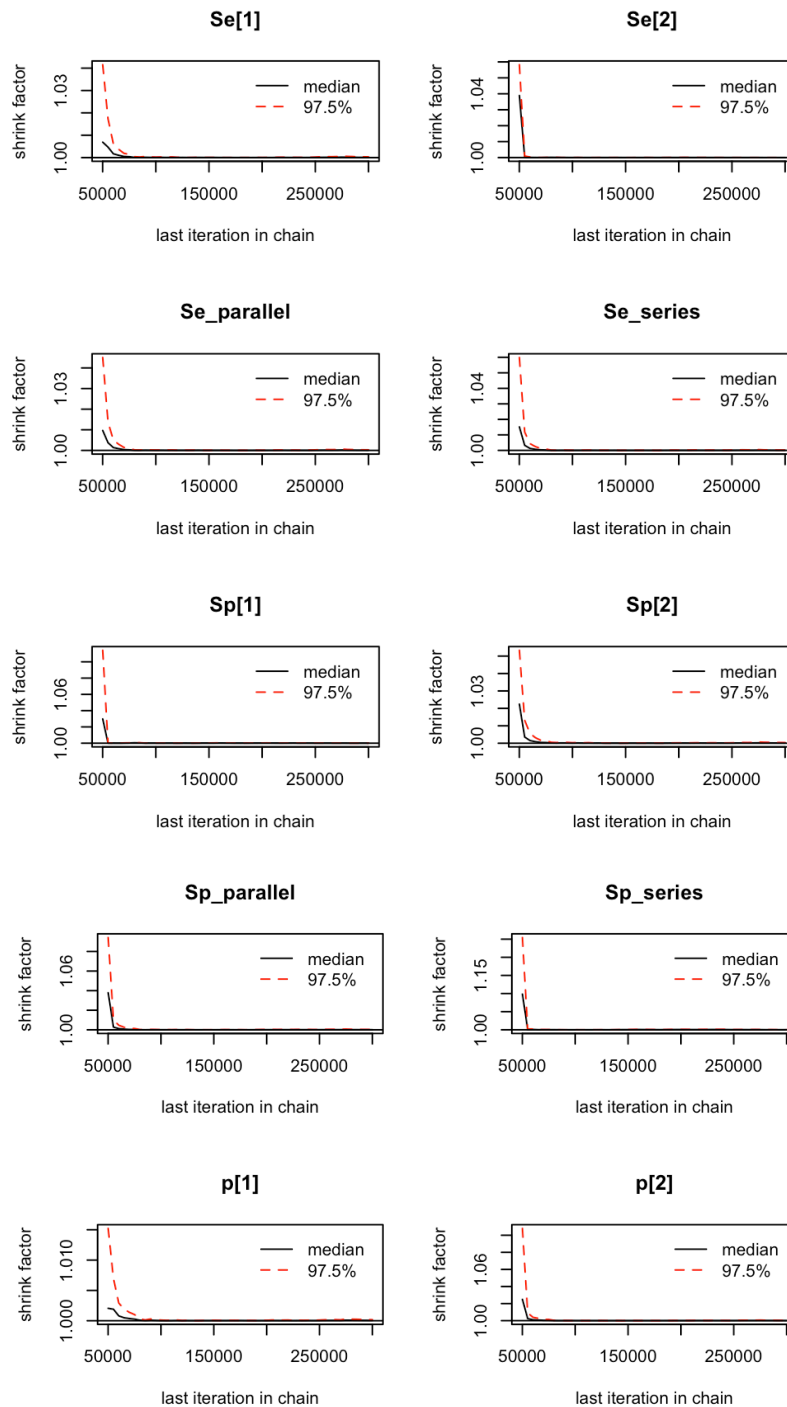
**Figure S9.4: Gelman-Rubin diagnostic plots showing convergence for each parameter of the caprine model with a uniform prior set and under conditional independence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Caprine model: Model with relaxed prior set and conditional dependence



Figure S9.5: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a caprine model under conditional dependence showing a relaxed prior set* (grey bars) and posterior estimates (red bars)
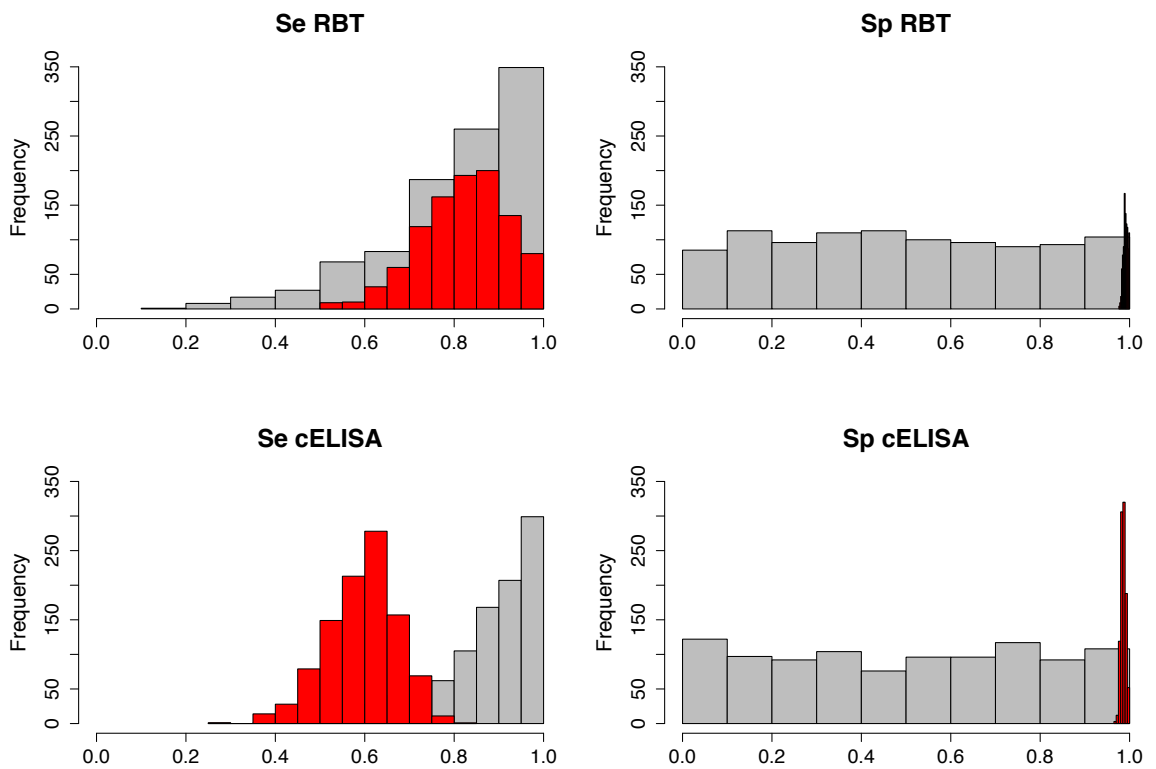
*relaxed prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(1,1)

**Figure S9.6: Gelman-Rubin diagnostic plots showing convergence for each parameter of the caprine model with a relaxed prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Caprine model: Model with relaxed prior set and conditional independence



Figure S9.7: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a caprine model under conditional independence showing a relaxed prior set* (grey bars) and posterior estimates (red bars)
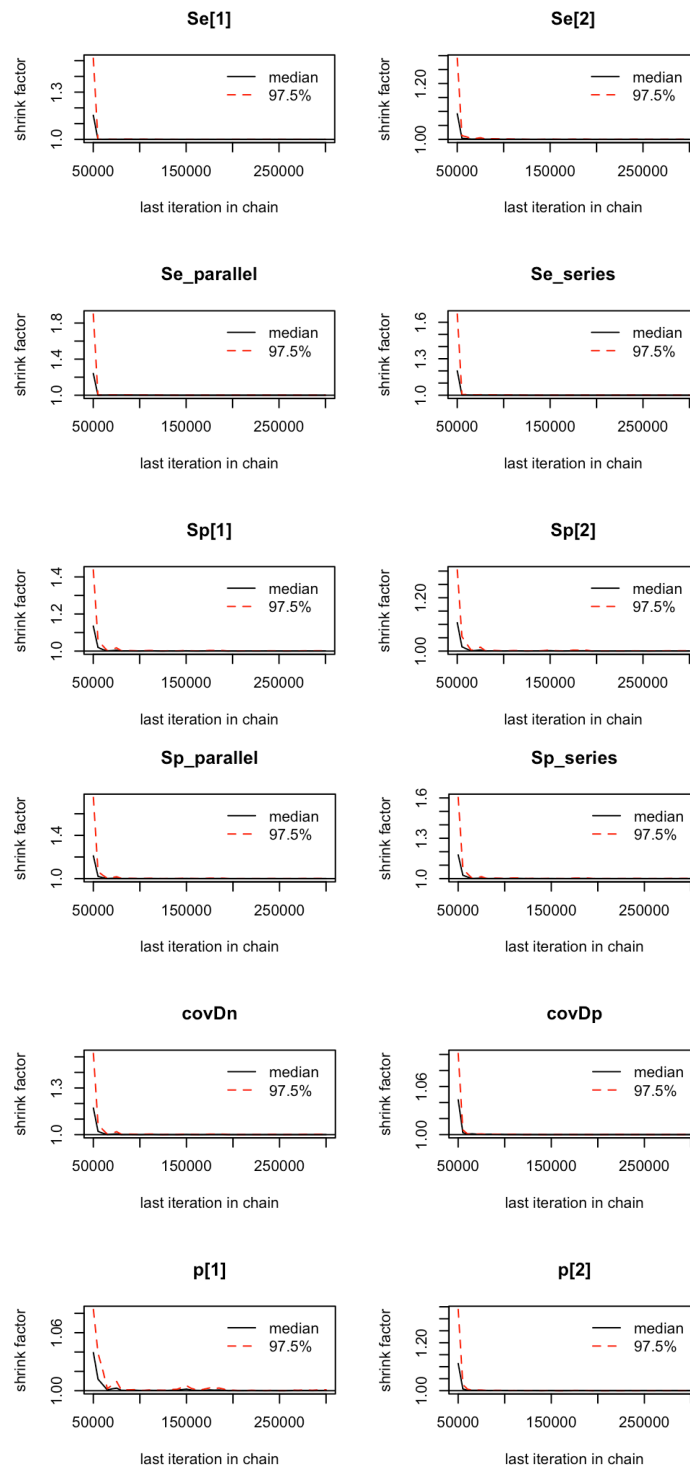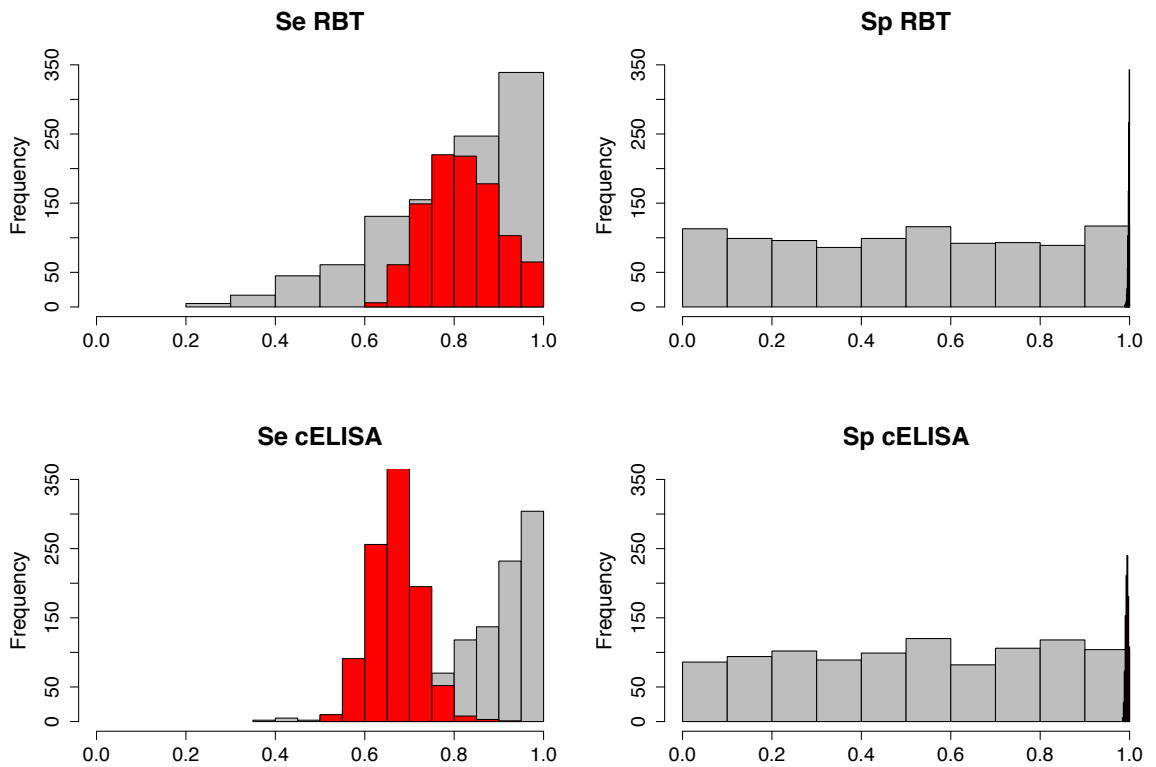*relaxed prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(1,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(1,1)
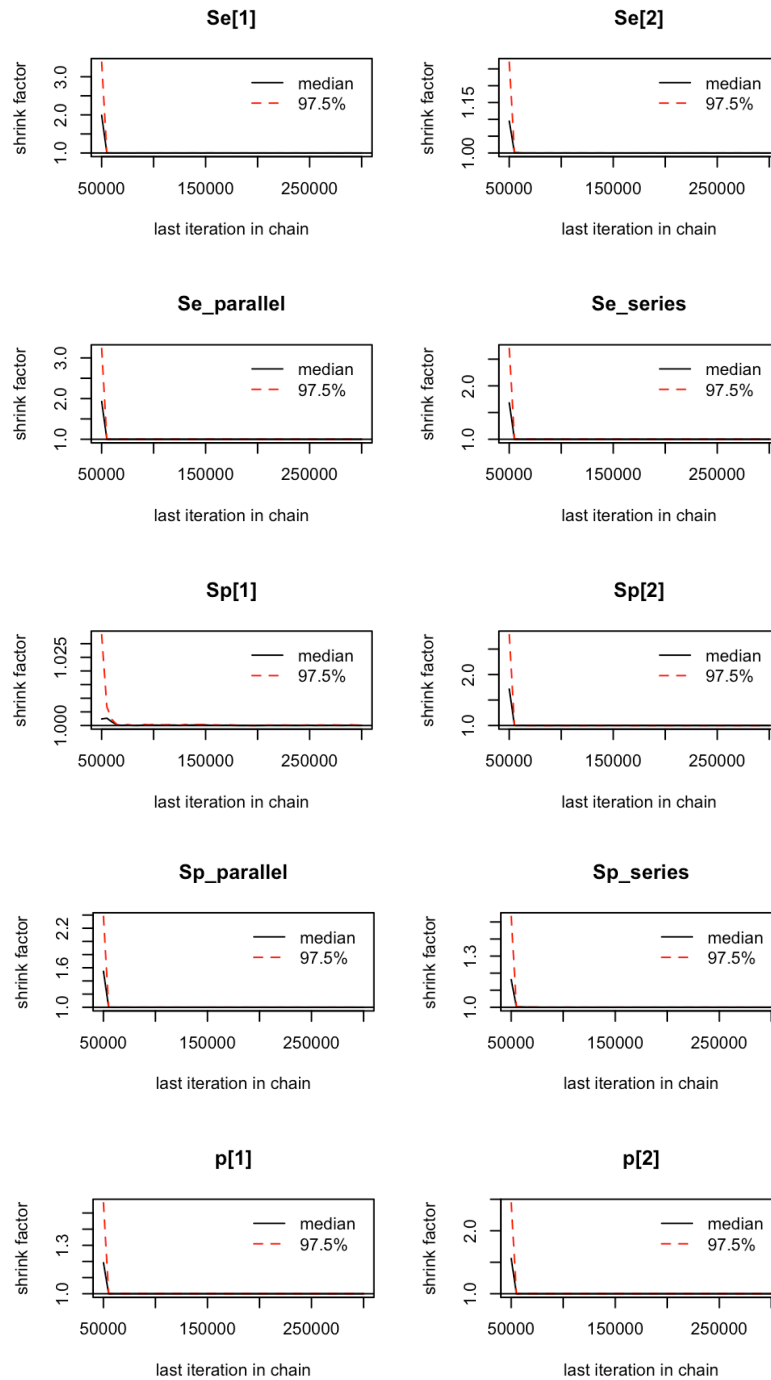
**Figure S9.8: Gelman-Rubin diagnostic plots showing convergence for each parameter of the caprine model with a relaxed prior set and under conditional independence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Caprine model: Model with strict prior set and conditional dependence



**Figure S9.9: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a caprine model under conditional dependence showing a strict prior set\* (grey bars) and posterior estimates (red bars)**
\*strict prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(2,1)
Se cELISA ~ dbeta(7,1)
Sp cELISA ~ dbeta(104,1)
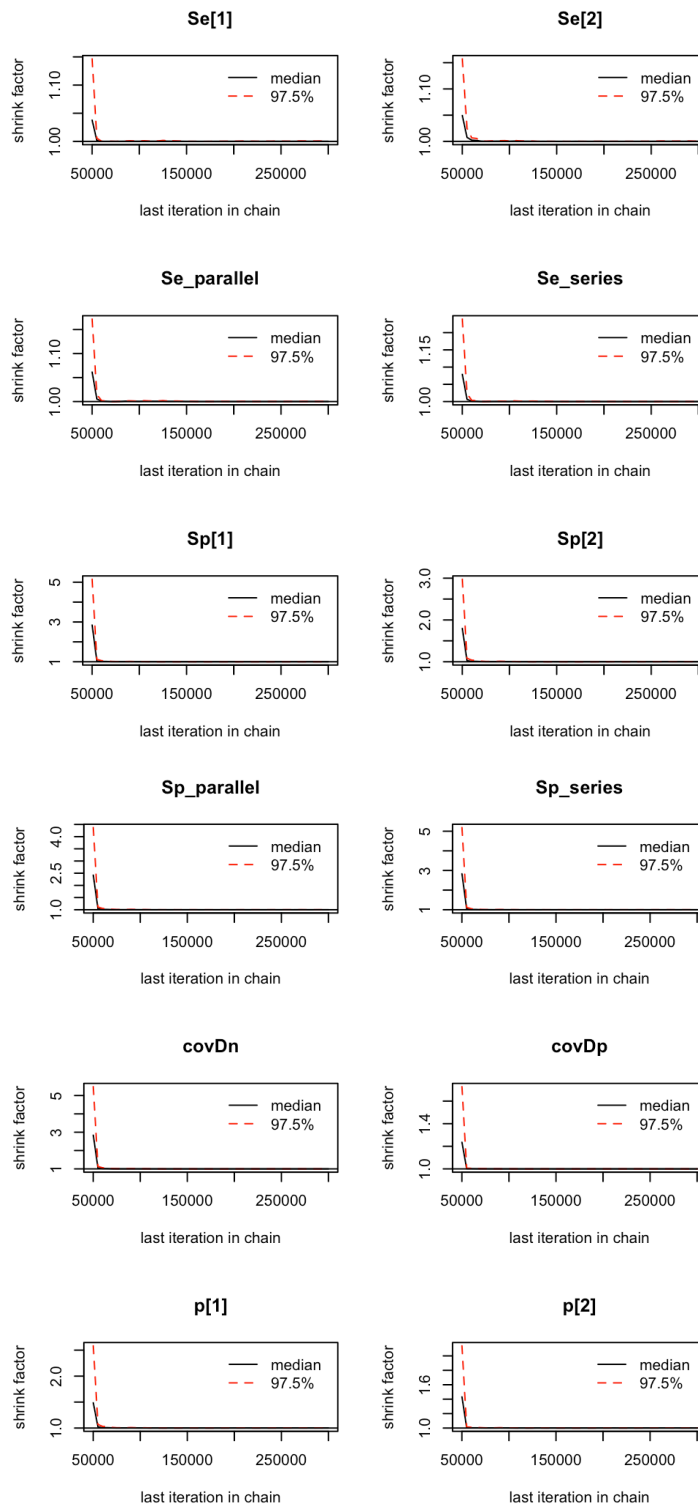
**Figure S9.10: Gelman-Rubin diagnostic plots showing convergence for each parameter of the caprine model with a strict prior set and under conditional dependence**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

# Caprine model: Model with strict prior set and conditional independence



Figure S9.11: Frequency plots for sensitivity (Se) and specificity (Sp) parameters of the Rose Bengal plate test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) for a caprine model under conditional independence showing a strict prior set* (grey bars) and posterior estimates (red bars)

*strict prior set:
Se RBT ~ dbeta(4,1)
Sp RBT ~ dbeta(2,1)
Se cELISA ~ dbeta(7,1)
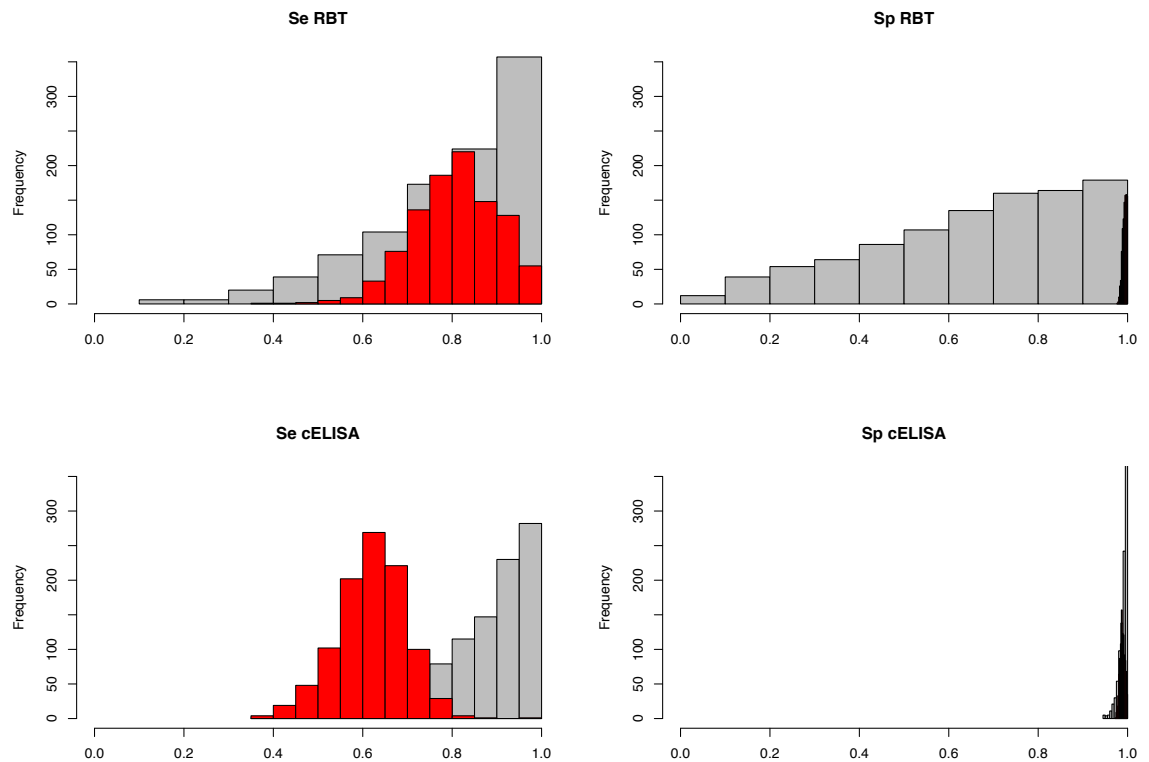Sp cELISA ~ dbeta(104,1)
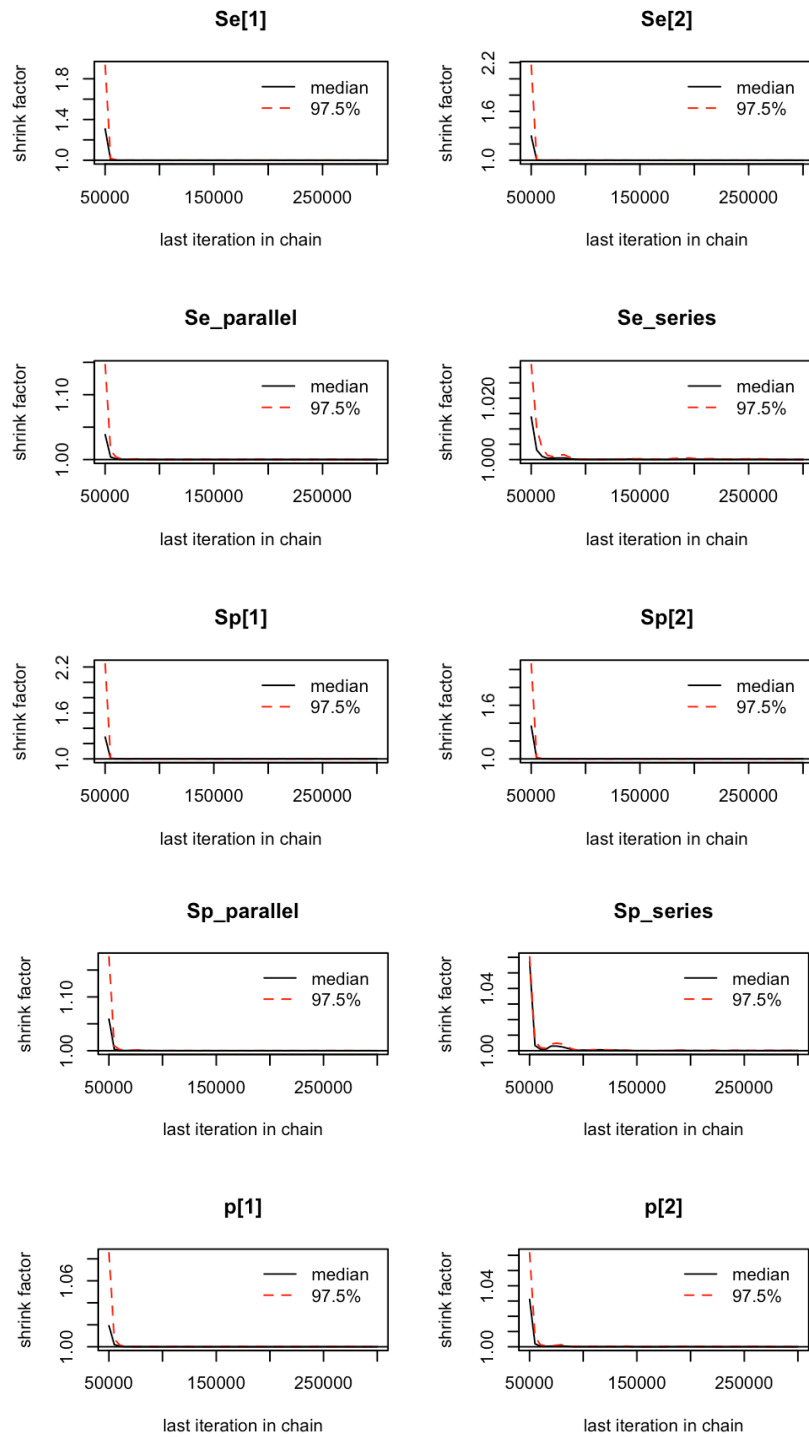
**Figure S9.12: Gelman-Rubin diagnostic plots showing convergence for each parameter of the caprine model with a strict prior set and under conditional independence***, Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

**Appendix 10: Caprine final model diagnostic plots**
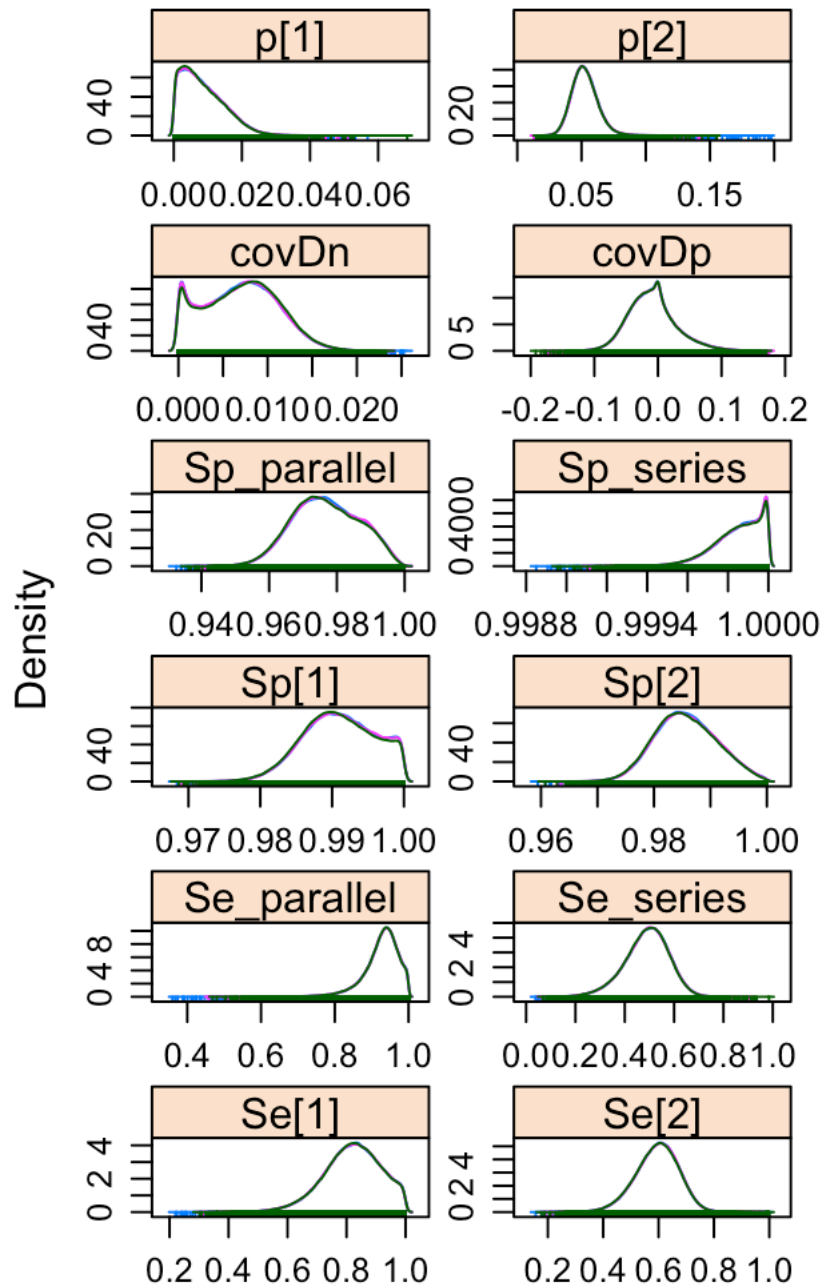
**Figure S10.1: Density plots for each parameter of the final caprine model**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

**Figure S10.2: Gelman-Rubin diagnostic plots showing convergence for each parameter of the final caprine model**, *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*

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**Figure S10.3: Trace plots showing MCMC chain convergence for each parameter of the final caprine model,** *Se[1] is sensitivity of the Rose Bengal plate test (RBT), Se[2] is sensitivity of the competitive enzyme-linked immunosorbent assay (cELISA), Sp[1] is specificity of RBT, Sp[2] is specificity of cELISA, Se_series is sensitivity of RBT and cELISA run in-series, Sp_series is specificity of RBT and cELISA run in-series, Se_parallel is sensitivity of RBT and cELISA run in-parallel, Sp_parallel is specificity of RBT and cELISA run in-parallel, covDn is covariance when animal is disease negative, covDp is covariance when animal is disease positive, p[1] is prevalence in a non-exclusive pastoralist population and p[2] is prevalence in an exclusive pastoralist population*
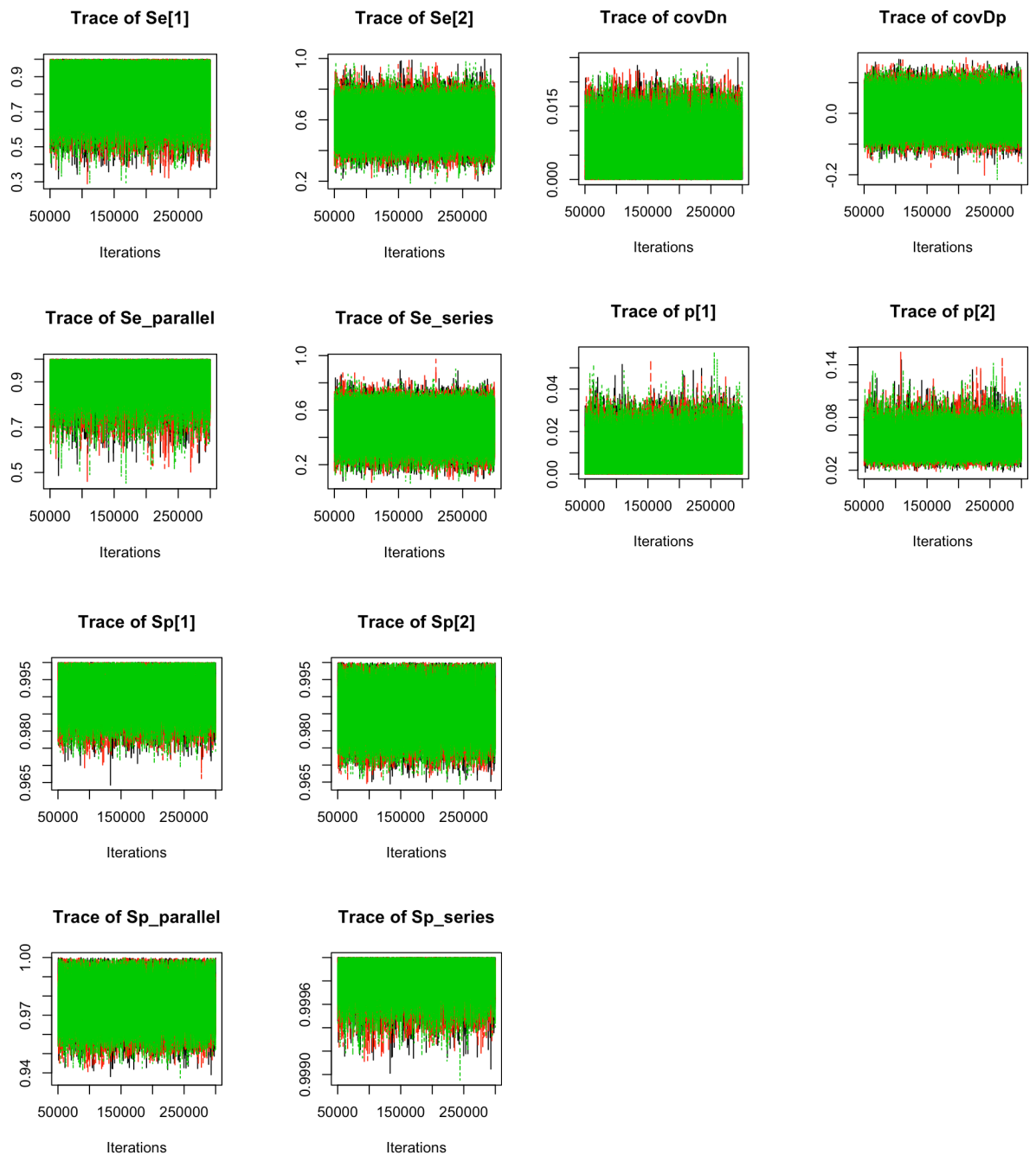
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# Appendix 11: Bayesian latent class model code: Caprine final model with conditional dependence

**# Set up data for JAGS**
```
pop <- t(matrix(with(data, table(rbt, celisa, population),
              dnn=c("rbt", "celisa", "population")), 4,2))
n.pop = 2
n = apply(pop,1,sum)
```

**# JAGS model**
```
# [1] is RBT
# [2] is cELISA
# Tests in order  -- / +- / -+ / ++

cat("model{
   for (i in 1:n.pop){
   pop[i, 1:4] ~ dmulti(par[i, 1:4], n[i])
   p[i] ~ dunif(0, 0.49)
   par[i,4] <- p[i]*   (Se[2] * Se[1] + covDp) + (1-p[i])*((1-Sp[2])*(1-Sp[1]) + covDn)
   par[i,3] <- p[i]* ((1-Se[2])* Se[1] - covDp) + (1-p[i])*((Sp[2])*(1-Sp[1]) - covDn)
   par[i,2] <- p[i]*   (Se[2] * (1-Se[1]) - covDp) + (1-p[i])*((1-Sp[2])*(Sp[1]) -
covDn)
   par[i,1] <- p[i]* ((1-Se[2])* (1-Se[1]) + covDp) + (1-p[i])*((Sp[2])*(Sp[1]) +
covDn)
   }

   ls <- (Se[1]-1)*(1-Se[2])
   us <- min(Se[1],Se[2]) - Se[1]*Se[2]
   lc <- (Sp[1]-1)*(1-Sp[2])
   uc <- min(Sp[1],Sp[2]) - Sp[1]*Sp[2]
   rhoD <- covDp / sqrt(Se[1]*(1-Se[1])*Se[2]*(1-Se[2]))
   rhoDc <- covDn / sqrt(Sp[1]*(1-Sp[1])*Sp[2]*(1-Sp[2]))

   Se[1] ~ dbeta(4,1)
   Sp[1] ~ dbeta(1,1)
   Se[2] ~ dbeta(7,1)
   Sp[2] ~ dbeta(1,1)

   # To get Serial and Parallel Ses and Sps
   Se_series <- Se[1] * Se[2]
   Se_parallel <- 1 - (1 - Se[1]) * (1 - Se[2])
   Sp_series <- 1 - (1 - Sp[1]) * (1 - Sp[2])
   Sp_parallel <- Sp[1] * Sp[2]
   covDn ~ dunif(lc, uc)
```

```
        covDp ~ dunif(ls, us)

        }", file="mod.jag")
```

**# Initial values for the three chains**
```
modelInit1 <- list(Se=c(0.7,0.99),  Sp=c(0.3,0.99),  p=c(0,0.1))
modelInit2 <- list(Se=c(0.3,0.8), Sp=c(0.15,0.7),  p=c(0.01,0.25))
modelInit3 <- list(Se=c(0.6,0.95),  Sp=c(0.4,0.90),  p=c(0.02,0.49))
INI <- list(modelInit1, modelInit2,modelInit3)
```

**# Compile model components**
```
M <- jags.model(data=list(pop=pop,n=n, n.pop=n.pop), inits=INI, n.chains=3,
n.adapt= 50000, file="mod.jag")
```

**# Run the model with 50,000 burn-in and a further 250,000 iterations and thinning every 100th iteration**
```
R <- coda.samples(M, c("Se", "Sp", "p", "Se_series", "Se_parallel", "Sp_series",
"Sp_parallel"), n.iter=250000, n.thin=100)
```

**# Check model deviance information criterion (DIC)**
```
dic.samples(M, n.iter=250000, n.thin=100, type="pD")
```

**# Check model convergence**
```
densityplot(R)
gelman.diag(R, multivariate = FALSE)
gelman.plot(R)
traceplot(R)
```

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